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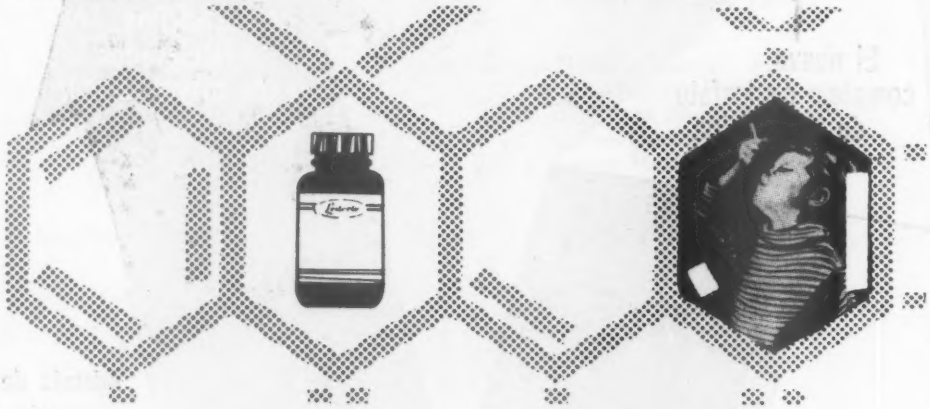

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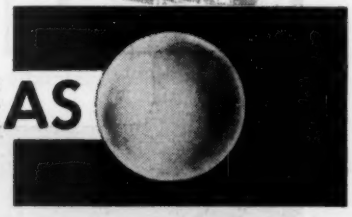

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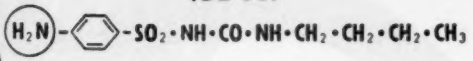

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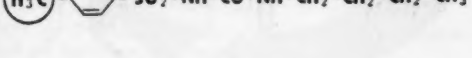

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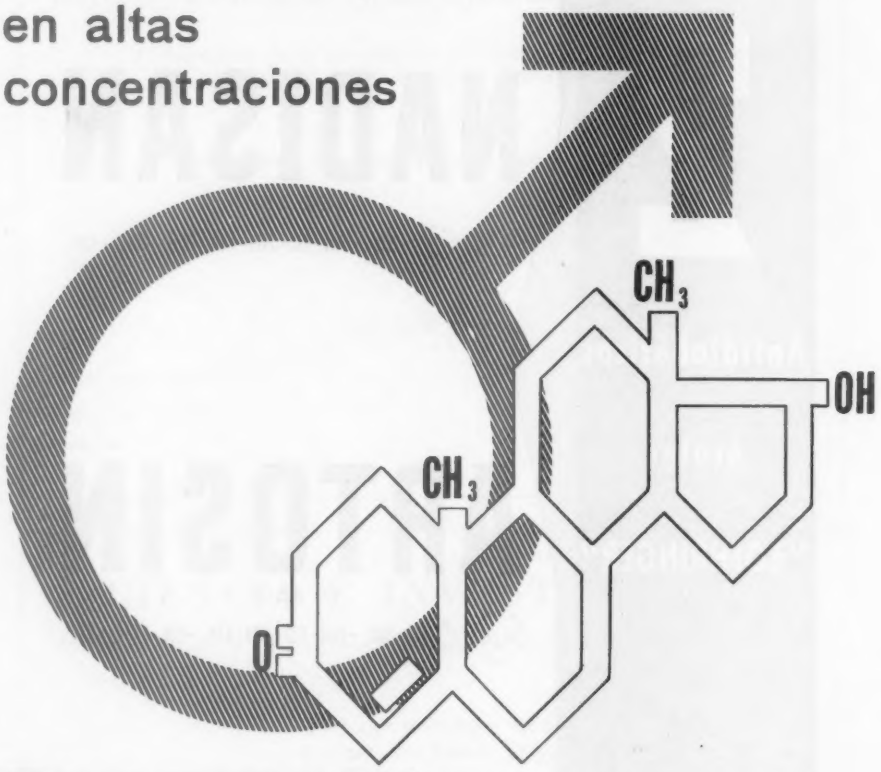

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The official program of the XXI International Congress of Physiological Sciences includes 12 symposia (three lectures each), 16 special lectures and 102 sessions in which 918 communications will be reported (all communications received beyond this number whill be read by title).

AUGUST 1959

Sunday 9 (10 A.M.) OPENING MEETING

	Communications	*Special Lectures*	*Symposia*
Monday 10	9 to 11.15 A.M.	11.45 to 12.35 A.M.	3 to 5.30 P.M.
Tuesday 11	9 to 11.15 A.M.	11.45 to 12.35 A.M.	3 to 5.30 P.M.
Wednesday 12	9 to 11.15 A.M.	11.45 to 12.35 A.M.	free
Thursday 13	9 to 11.15 A.M.	11.45 to 12.35 A.M.	3 to 5.30 P.M.
Friday 14	9 to 11.15 A.M.	11.45 to 12.35 A.M.	3 to 5.30 P.M.
Saturday 15	9 to 11.15 A.M.	11.40 A.M. CLOSING MEETING	

Communications: 17 simultaneous meetings with 9 communications each, per day: 153 communications per day = 918 communications. (10 minutes for each exposition and 5 additional minutes for discussion.)
Summaries: 300 words.

Special Lectures: 3 or 4 simultaneous sessions per day: 5 days = 16 Special Lectures. (30 minutes for each exposition and 20 additional minutes for discussion.)
Summaries: 3.500 words.

Symposia: 3 simultaneous symposia per day: one chairman and three lectures for each symposium = 36 lectures. (30 minutes for each exposition and 20 additional minutes for discussion.)
Summaries: 3.500 words.

Simultaneous translations will be provided for Special Lectures and Symposia. Lectures delivered in English will be translated into Spanish and viceversa. Lectures delivered in French will be translated into English.

ALL SUMMARIES MUST REACH THE SECRETARY OFFICE
BEFORE FEBRUARY 1st, 1959

Subjects of the Communications

Papers on Physiology and Pharmacology will be delivered at the Congress sessions on subjets dealing with: general physiology, general pharmacology, cellular physiology and pharmacology, blood and body fluids, circulation, respiration, digestion, absorption and excretion, kidney function, sweat secretion, permeability, ionic transport, electrophysiology nutrition, metabolism, carbohydrate, protein, fat and sterols metabolism, water and electrolytes metabolism, vitamins, animal heat and thermoregulation (hypothermia, hyperthermia), internal

secretions, reproduction, applied physiology and pharmacology, organs of movement, muscle and exercise, nervous system, peripheral, central, autonomic, synaptic transmission, neurochemistry, neuropharmacology, sense organs, and others.

Monday, August 10th, 1959

(11.45 to 12.35 A.M.)

SPECIAL LECTURES

Room 1 Chairman: *R. Courrier*

A. JOST: "Le role des hormones foetales dans le métabolisme du foetus"

Room 2 Chairman: *C. F. Schmidt*

A. HURTADO: "La función respiratoria en la altura".

Room 3 Chairman: *J. C. Eccles*

E. DE ROBERTIS: "Histofisiología de la sinapsis".

(3 to 5.30 P.M.)

SYMPOSIA

Room 1 *Function of the Pregnant Uterus*

Chairman: *G. W. Corner*

R. CALDEYRO BARCIA: "Contractilidad del útero grávido y factores que lo controlan".

R. COURRIER: "Endocrinologie de l'utérus gravide".

A. CSAPO: "The molecular aspects of function and regulation in the pregnant and parturient myometrium".

Room 2 *Mechanism of Adaptation to Hypoxia*

Chairman: *A. Hurtado*

A. B. OTIS: "Physiological adaptation to the chronic hypoxia associated with right to left shunts of the circulation. A comparison with the hypoxia of high altitudes".

N. PACE: "Adaptations of sojourners at high altitude".

B. REYNAFARJE: "Estudios de química tisular en la hipoxia".

Room 3 *Synaptic Transmission*

Chairman: *Sir L. Brown*

S. V. ANITCHKOV: "Pharmacology of the central cholinergic synapses".

S. W. KUFFLER: "Synaptic inhibition".

P. WASER: "Autoradiographic investigations on receptors of the endplate".

Tuesday, August 11th, 1959

(11.45 to 12.35 P.M.)

SPECIAL LECTURES

Room 1 Chairman: *J. V. Luco*

M. R. COVIAN: "Efectos de la hemidecorticación sobre las funciones vege tativas".

Room 2 Chairman: *S. W. Kuffler*

A. ROSENBLUETH: "El período refractario funcional de las fibras excitables".

Room 3 Chairman: *W. O. Fenn*

R. F. RUSHMER: "Neural control of the heart in intact dogs".

(3 to 5.30 P.M.)

SYMPOSIA

Room 1 *Central Inhibition*

Chairman: *P. S. Kupalov*

A. FESSARD: "Les processus de base de l'inhibition nerveuse centrale".
I. G. BERITOFF: "On the origin of the central inhibition".
H. W. MAGOUN: "Central inhibition and the reticular system".

Room 2 *Vasoactive Polypeptides*
Chairman: *H. Croxatto*
L. T. SKEGGS: "Hypertensin and allied substances".
M. ROCHA E SILVA: "Bradykinin and allied substances".
H. B. VAN DYKE: "Vasopressin".

Room 3 *Hypothermia*
Chairman: *E. F. Adolph*
CH. KAYSER: "Hibernation et hypothermie artificielle".
P. F. SCHOLANDER: "Supercooling and freezing in poikilotherms".
A. U. SMIHT: "Survival of mammals at body temperatures above and below 0°C".

Wednesday, August 12th, 1959

(11.45 to 12.35 A.M.)

SPECIAL LECTURES

Room 1 Chairman: *Z. M. Bacq*
R. GERSCHMAN: "Oxygen offects in biological systems".

Room 2 Chairman: *D. Bennati*
P. RIJLANT: "Electrogenèse globale du Coeur".

Room 3 Chairman: *R. Granit*
T. TOMITA: "Study on electrical activities in the retina with penetrating microelectrodes".

Room 4 Chairman: *C. Bykov*
A. A. VOLOCHOFF: "Le développement des réflexes inconditionnel et conditionnel et leur interaction dans l'ontogenèse".

(3 to 5.30 P.M.)

F R E E

Thursday, August 13th, 1959

(11.45 to 12.35 A.M.)

SPECIAL LECTURES

Room 1 Chairman: *A. Fessard*
B. K. ANAND: "Higher nervous control over food intake".

Room 2 Chairman: *U. S. von Euler*
C. CHAGAS: "Mecanismos fisiológicos na descarga de peixes eletricos".

Room 3 Chairman: *M. Rocha e Silva*
V. ERSPARMER: "The biological significance of 5 HT in the living organism. Present status of the problem".

(3 to 5.30 P.M.)

S Y M P O S I A

Room 1 *Patterns of Connection in the Spinal Cord*
Chairman: *Ch. McC Brooks*
J. C. ECCLES: "Monosynaptic excitatory patterns and the central connections of Group 1 afferent fibers from muscle".

R. GRANIT: "Circuit analysis of postural reflexes and the relative significance of alpha and gamma motoneurones".

A. LUNDBERG: "Integrative significance of patterns of connections made by muscle afferents in the spinal cord".

Room 2 *Radiation Protection and Recovery*

Chairman: *A. Hollaender*

Z. M. BACQ: "Action of radioprotectors in mammals".

D. W. H. BARNES: "The treatment of lethally irradiated mice: The radiation chimaera".

CH. C. CONGDON: "Immunologic problems in tissue transplantation for recovery from radiation injury".

Room 3 *Adrenaline and Noradrenaline*

Chairman: *Sir H. H. Dale*

H. BLASCHKO: "The origin and metabolism of adrenaline and noradrenaline"

U. S. VON EULER: "The physiological role of adrenaline and noradrenaline".

J. MALMEJAC: "Nature et régulation de la sécrétion médullo-surrénale".

Friday, August 14th, 1959

(11.45 to 12.35 A.M.)

SPECIAL LECTURES

Room 1 Chairman: *C. Heymans*

D. BOVET: "Pharmacologie de la circulation cérébrale".

Room 2 Chairman: *E. Lundsgaard*

E. F. ADOLPH: "Physiological regulation of body water content and its evolution".

Room 3 Chairman: *A. von Muralt*

H. H. WEBER: "Muscle physiology".

(3 to 5.30 P.M.)

SYMPOSIA

Room 1 *Physiological Contributions from Studies on Central Acting Drugs*

Chairman: *D. Bovet*

H. GASTAUT: "Neuroleptiques".

M. MONNIER: "Stimulants hallucinogènes et analeptiques du système nerveux central".

J. E. P. TOMAN: "Mechanisms of actions of convulsant and anticonvulsant drugs".

Room 2 *Ionic Transmission Across Membranes*

Chairman: *W. O. Fenn*

E. J. CONWAY: "The energetics of ion movements across membranes".

A. L. HODGKIN: "Ionic movements during activity and recovery in excitable tissues".

H. H. USING: "Active sodium transport and its relation to other physiological phenomena".

Room 3 *Life in the Space*

Chairman: *E. Braun-Menéndez*

A. POKROWSKY: "Etudes médico-biologiques de la vie dans l'espace, dans les fusées et les spoutniks artificiels de la terre, entreprises au courant de l'année internationale géophysique".

(United States scientists: name and title to be reported on later date.)

STUDIES ON A VASODILATOR POLYPEPTIDE PRESENT IN PURIFIED HYPERTENSIN PREPARATIONS

J. C. Fasciolo, K. A. Halvorsen, J. E. Itoiz
With the technical assistance of students: M. M. Puebla, A. Binia, E. Zangheri, F. E. Alonso
(Departamento de Fisiología - Facultad de Ciencias Médicas Universidad Nacional de Cuyo - Mendoza)
and
A. C. Paladini
(Instituto de Fisiología - Facultad de Ciencias Médicas Universidad de Buenos Aires - Buenos Aires)

In the course of an experiment in which the hind leg of a dog was perfused with a modified Schuster-Dale pump, a purified hypertensin preparation was injected in the cannula leading to the femoral artery. Instead of the expected vasoconstriction, it was observed that our hypertensin preparation was strongly vasodilator in the perfused circuit, while producing its usual pressor action when the main circuit was reached. We decided to investigate the cause of such anomalous finding.

Material and methods

The hypertensin preparations were obtained as follows: a crude renin preparation ([3]), was incubated with ox globulin obtained by precipitation of the plasma with ammonium sulphate between 1.3 and 2.2 M. After an incubation of 15 to 20 minutes at 37° C the mixture was precipitated with 3 volumes of alcohol, filtered and concentrated in vacuo. The concentrate was extracted with ethyl ether at pH 1.5 and the ether discarded. The aqueous layer was freed of ether by evaporation, saturated with sodium chloride and extracted several times with 2-butanol. The residue obtained by evaporating the butanol was submitted to countercurrent distribution in the system 2-butanol-0.1 N HCl (1/1). The contents of the tubes with peak activity were pooled, evaporated to dryness and the residue extracted with glacial acetic acid. Low temperature evaporation of the acetic acid gave the preparation of hypertensin

The authors wish to express their appreciation to Dr. M. Rocha e Silva who supplied the bradykinin and to Drs. L. T. Skeggs and J. R. Kahn who made available their pure hypertensins.

This work was supported by a grant from E. R. Squibb and Sons Argentina S. A.

Received for publication, May 30, 1958.

employed. Countercurrent distribution studies ([13]), have demonstrated that these preparations are frankly heterogeneous.

Mongrel dogs anesthetized with sodium nembutal (40 mg/kg) were employed for the test. According to the circumstances one hind leg or a kidney were perfused, using heparin (10 mg/kg), as an anticoagulant. The technique of the perfusion has been already described ([4]). The general scheme was as follows:

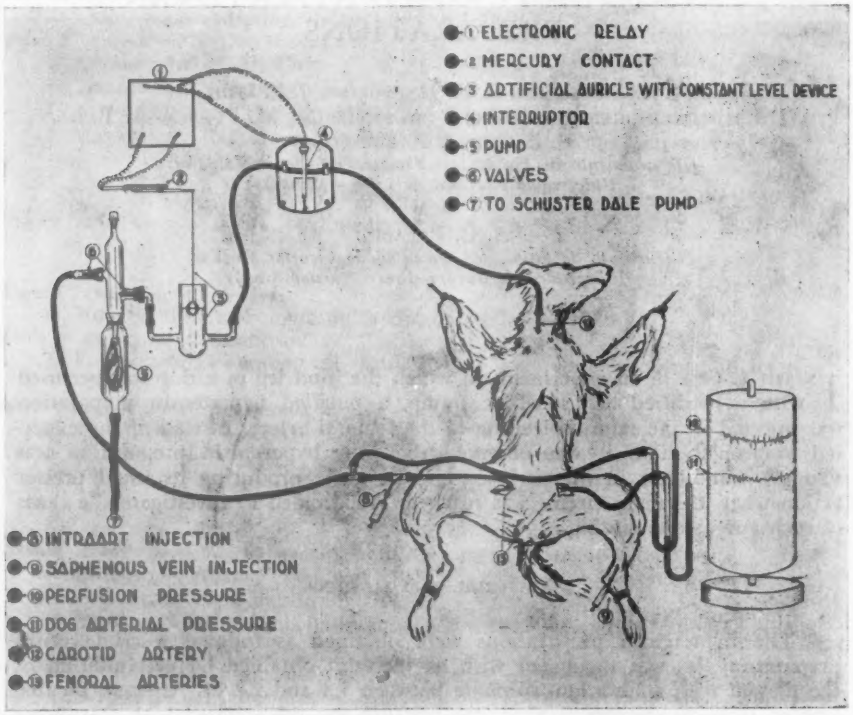

FIG. 1.—*SCHEME OF A PERFUSION EXPERIMENT. The blood comes from the carotid artery, passes through the interruptor to the artificial auricle and the pump, which forces it into the femoral artery. The perfusion pressure and the arterial pressure are registered simultaneously.*

one of the carotid arteries was cannulated and connected by a rubber tube to a small reservoir (artificial auricle) leading to the pump (fig. 1). The level of the blood in the artificial auricle was kept constant by an electronic relay. The Schuster-Dale pump forced the blood into the artery and since its output was kept constant, any change in the perfusion pressure should be due to a corresponding change in the vascular resistance of the circuit supplied by the pump. The blood, pumped out of the perfused organ, drained by the venous system into the dog's general circulation, and after being arterialized it flowed

into the arterial tree and then went back to the pump. In this way a vasoactive substance injected into the perfusing circuit, acts first on the perfused system and then on the general circulation. On the other hand, if the injection is given into a vein the vasomotor action is first seen in the general circuit and later in the perfused one.

Rat blood pressure. White rats weighing about 300 gr., anesthetized with nembutal (5 mg/kg), were used. A small glass cannula was introduced into the carotid artery and connected by a polyethylene tubing to a small mercury manometer filled with a direct ink recording system. Heparin, (10 mg), was used as an anticoagulant. The injections were given by a polyethylene tubing introduced into the jugular vein.

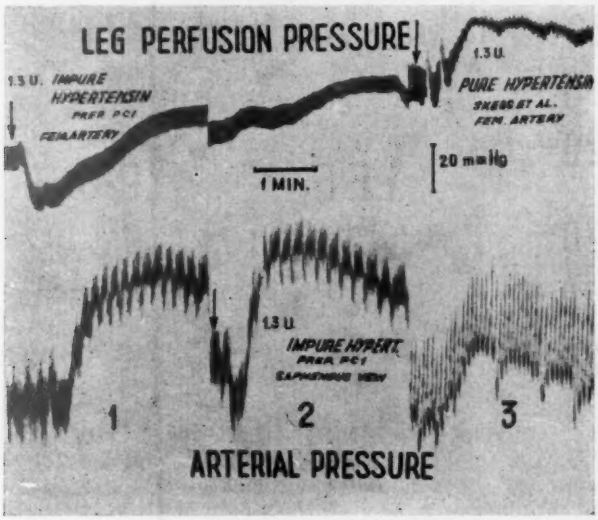

FIG. 2.—*SIMULTANEOUS REGISTRATION OF LEG PERFUSION PRESSURE AND ARTERIAL PRESSURE IN A DOG.*

1) Hypertensin preparation PC1 injected in the perfusion stream, produces a depression of the perfusion pressure, followed by a rise in the arterial pressure when the substance reaches the main circulation.

2) PC1 injected in the saphenous vein produces a small initial depression of the arterial pressure followed immediately by a steep rise. The perfusion pressure shows a delayed smooth rise.

3) Pure hypertensin injected in the perfusion stream produces a rise without any depression in both circuits.

RESULTS

Vascular effects of hypertensin preparation PC1. When hypertensin preration PC 1 was injected in the arterial circuit of a perfused leg or kidney, a fall in the perfusion pressure occurred showing that a vasodilatation was produced. This was also true with other hypertensins, prepared by the same method. Figure 2 shows the result of a typical experiment. 1.3 pressor units

of preparation PC 1 injected in the femoral artery of a perfused leg, produced a fall in the perfusion pressure. When the injected substance reaches the general circuit the arterial pressure rises (fig. 2, 1, below); that is to say that the same hypertensin preparation has a vasodilator effect on the perfused leg while producing a vasoconstriction on the main circuit.

The same amount, 1.3 pressor units injected in the saphenous vein, caused

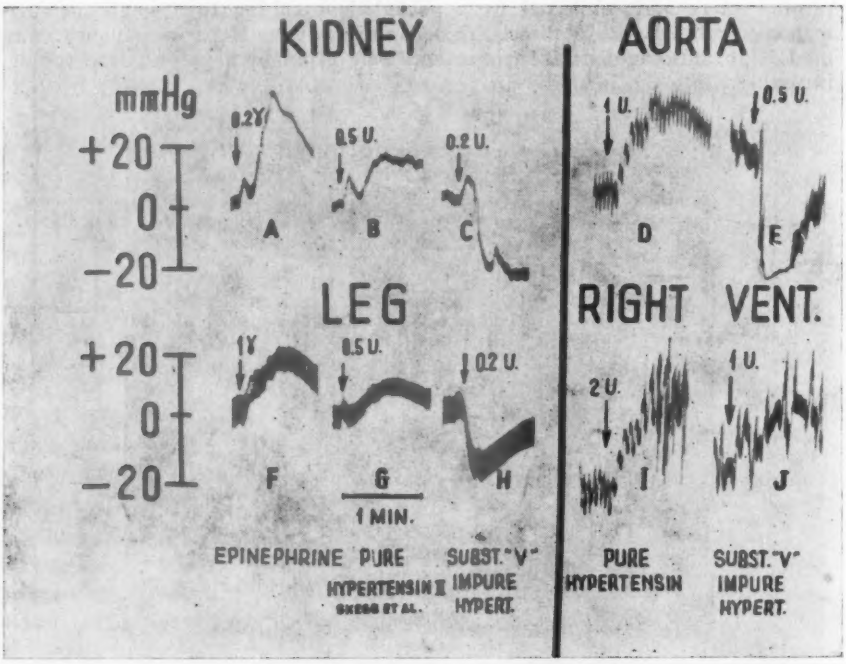

FIG. 3. — *Left: PRESSURES OF THE ARTERIAL CIRCUITS OF PERFUSED KIDNEY AND LEG. Epinephrine (A, F) and pure hypertensin (B, G) produce a rise in both circuits. Substance V (PCl), on the other hand (C, H), produce a fall in the pressure both in the kidney and in the leg.*

Right: ARTERIAL PRESSURE OF AN ANESTHETIZED DOG. Pure Hypertensin raises the arterial pressure either when injected into the aorta (D) or the right ventricle (I), while the PCl preparation is depressor when injected into the aorta (E) and pressor (J) when injected into the right ventricle.

an immediate small depression of the blood pressure, followed by a sharp increase (fig. 2, 2, below). The perfusion pressure of the leg (fig. 2, 2, above) showed a delayed, steady rise, not preceded by any depressor effect.

The results were quite different when pure hypertensin was used (*). In this case the intraarterial injection of 1.3 units was followed by a rise of the perfusion pressure (fig. 2, 3, above) without any depressor effect. When the

(1) Kindly supplied by Drs. Skeggs and Kahn.

injected substance reaches the main circuit it also produces a rise in the arterial pressure (fig. 2, 3, below).

Figure 3 shows the results obtained with perfused kidneys. Epinephrine injected intraarterially (0.2 γ) caused a strong vasoconstriction (A). Pure hypertensin was also vasoconstrictor (B) but preparation PC 1 had a vasodilator effect (C). These results are similar to the ones obtained with the perfused

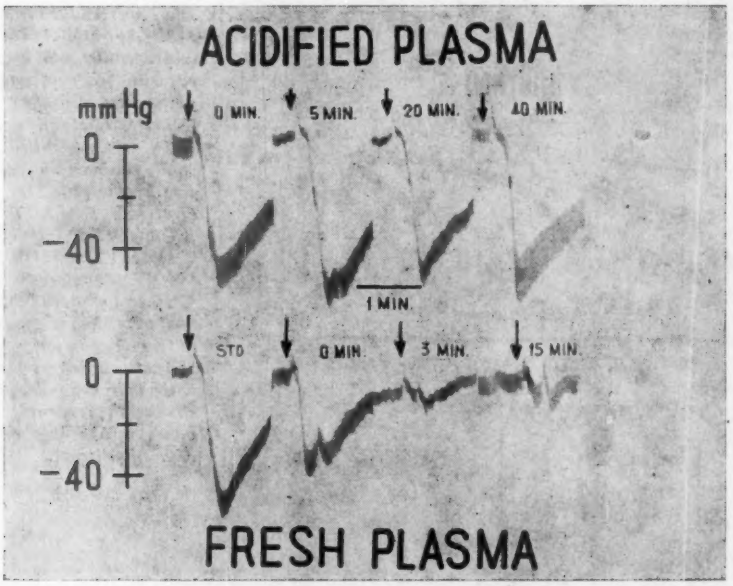

FIG. 4.—*INCUBATION OF PREPARATION PC1 WITH PLASMA. Three depressor units of PC1 (substance V) were incubated with 5 ml of plasma previously acidified (according to Leloir et al [10]), phosphate buffer and merthiolate solution, final volume 7, ml (above); and with unacidified plasma (below).*

leg, (F, G, H), with the difference that the kidney is more sensitive to vasoconstrictors. The injection of pure hypertensin into the aorta (D) or in the right ventricle was followed by a pressor effect. On the other hand, preparation PC 1 had a pressor effect when injected into the right ventricle (J) but caused a fall in the blood pressure, when the injection was given into the aorta (E).

All these findings support the idea that our hypertensin preparations are contaminated by a vasodilator substance which is strong enough as to inhibit the vasoconstrictor action of hypertensin when injected intraarterially. The depressor substance, to which we shall refer from now on as substance "V", must be destroyed rapidly in the blood stream since in order to obtain vasodilatation the injection must be given intraarterially, the venous or ventricular injection inducing only a pressor response.

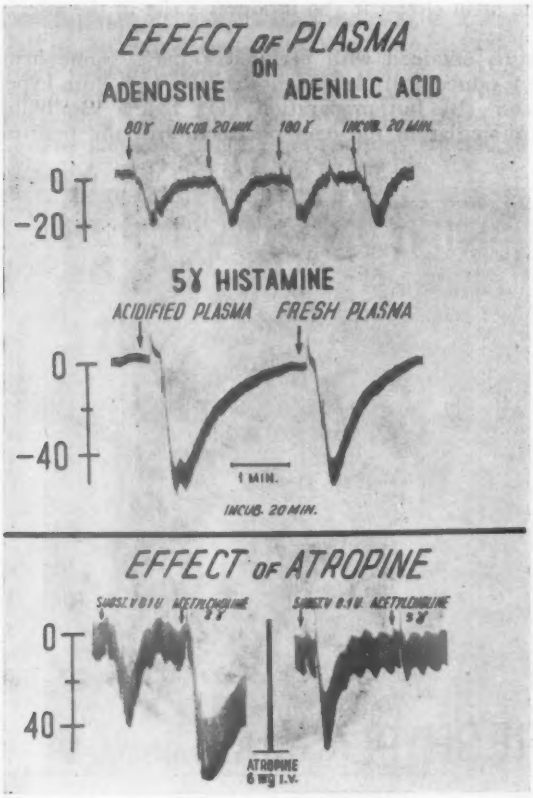

FIG. 5.—*LEG PERFUSION PRESSURE, INCUBATIONS OF ADENOSINE, ADENILIC ACID AND HISTAMINE WITH PLASMA. (Above). Adenosine and adenilic acid incubated with fresh plasma are not destroyed. (Middle) Histamine incubated with acidified and fresh plasma. No destruction is observed. (Below) EFFECT OF ATROPINE ON SUBSTANCE V AND ACETYLCHOLINE. Before the atropine treatment, substance V and acetylcholine produce a fall of the perfusion pressure; after injecting the dog with 6 mg atropine, acetylcholine depression is inhibited, while substance V depression is not.*

Destruction of substance "V" by the plasma. To test the hypothesis that substance "V" was destroyed by the blood, 3 pressor units of preparation PC 1 were incubated at 37° C with 5 ml of fresh dog's plasma, using merthiolate 1/10.000 as a preservative. The pH was kept about 7.4 with phosphate buffer, the total volume of the solution being 7.5 ml. The results (fig. 4) show that the destruction of "V" is almost complete after 3 m incubation. On the other hand, il the plasma is treated before incubation, according to Leloir et al ([10]) to destroy hypertensinase, (incubation at 37° and pH 4 for 30m), its ability to destroy substance "V" is completely lost. This finding supports the idea that substance "V" is destroyed in the plasma by enzymatic action.

Identification of the vasodilator substance. The differentiation of substance "V" from adenosine, adenilic acid, histamine or acetylcholine is easily made. Neither adenosine, nor adenilic acid or histamine are destroyed by plasma (fig. 5), which rapidly destroys substance "V". The vasodilator action of acetylcholine is completely inhibited by atropine, while the effect of substance "V" is unmodified (fig. 5). Angiodiactin ([7]) which produces a vasodila-

tion on the Läwen-Trendelenburg toad preparation constricted by epinephrine or hypertensin, was found to be without effect on the blood perfused organs.

Substance "V" appears to be related to a group of vasodilator substances of polypeptide nature, such as bradykinin ([15]), Kallidin ([8]), substance "P" ([5]), substance "U" ([1]), darmstoff ([20]) and kinin ([18]). We were able to compare substance "V" with bradykinin obtained through the courtesy of Professor Rocha e Silva.

Comparison between substance "V" and bradykinin. In order to compare the vasodilator action of both substances, a vasodilator unit was defined as the amount that will produce a decrease of 50 mm Hg in the perfusion pressure, when injected intraarterially into a leg preparation of a 10 kilogram dog, as described above.

The bradykinin preparation we used contained 1 vasodilator unit per 200 µg of dry substance. Hypertensin preparation PC1 contained 1 depressor unit in approximately 50 µg of dry substance.

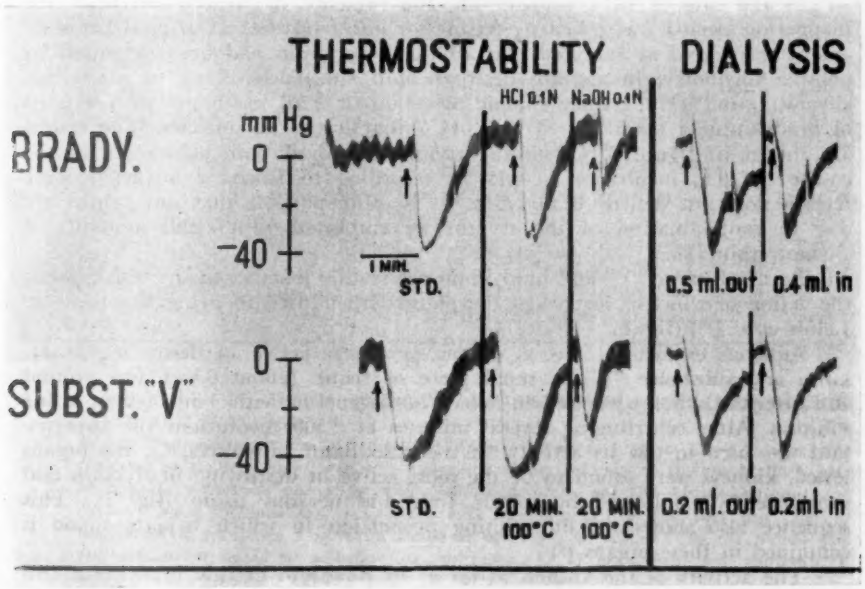

FIG. 6.—*(Left) THERMOSTABILITY OF BRADYKININ AND SUBSTANCE V. Substance V. 5 U. and bradykinin, 5 U., were dissolved in 2,5 ml of 0,1 N.HCl, and in 25 ml of 0,1 N.NaOH; after 20 minutes at 100° C they were neutralized and 0,5 ml of each were injected in the perfusion stream. It can be seen that both were resistant to the boiling with HCl, while most of the depressor action was lost in the basic medium.*

(Right) LEG PERFUSION PRESSURE. DIALYSIS OF BRADYKININ AND SUBSTANCE V. 2 U. bradykinin in 2 ml, and 4 U substance V in 2 ml saline, were dialized in a small cellophane bag against the same saline volume, for 40 hours at 4° — 5° C. The solutions outside and inside the cellophane bag were compared on the perfused leg, showing that an equilibrium had been attained.

Thermoestability. Figure 6 shows that neither bradykinin nor substance "V" were destroyed after 20 m boiling in acid solution (0.1 N H Cl) the depressor activity being the same as the controls. On the other hand, the activity of both substances was greatly reduced by boiling 20 minutes in an alkaline (0.1 N Na OH) medium.

Dialysis. Both bradykinin and substance "V" dialyse through cellophane membranes. Figure 6 shows that a nearly complete equilibrium is attained when the cellophane bags in which the substances were introduced, were kept in a small volume of distilled water for 40 hours at refrigerator temperature (about 5° C).

Enzymatic destruction. Crystalline pepsin does not destroy substance "V" or bradykinin. Figure 7 shows that the incubation of 5 vasodilator units of both substances with 1 mg of crystalline pepsin (final volumen 2,5 ml) at pH 1 for 30 minutes does not reduce their vasodilator action. This was also true when the incubation was prolonged up to 60 minutes (in this case the final volumen was 5 ml, that is to say that the enzyme concentration was halved).

In the case of PC 1 preparation, the presence of hypertensin after the incubation period was tested by its pressor effect on the rat arterial pressure, and it was found as expected that all the hypertensin had been destroyed by pepsin. Chymotrypsin rapidly destroys both substances. 1 mg of crystalline chymotrypsin (*) in a final volume of 2.5 ml or 5 ml incubated with 5 units of bradykinin or substance "V" at pH 7 for 30 and 60 minutes. The results are shown in figure 7. Crystalline trypsin destroyed both substances in the course of 30 m incubation at pH 7. According to Rocha e Silva ([17]) pure trypsin does not destroy bradykinin. It is quite possible that our results are due to contamination of the trypsin we employed with small amounts of chymotrypsin.

Both substance "V" and bradykinin are readily inactivated by fresh plasma, the action of which is lost when the plasma is acidified to pH 4, according to Leloir et al. ([10]) (fig. 8).

Aqueous extracts of several organs were very active in destroying bradykinin and substance "V". A small piece of tissue (about 0.5 g) was ground and afterwards homogeneized in a ball homogeneizer with 5 ml of 9 ‰ saline solution. After centrifuging several minutes at 2 500 revolutions the supernatant was used to test its activity on the vasodilator substances. Of the organs tested, kidneys were found to be the most active in destroying bradykinin and substance "V", followed by spleen, liver and nervous tissue (fig. 7). This sequence also shows the diminishing proportion in which hypertensinase is contained in these organs ([6]).

The activity of the kidney, as far as its power to destroy bradykinin and substance "V" is concerned, is remarkable. 2 mg of fresh cortex, completely destroys 4 units if both substances in 30 m, which is comparable with the action of 1 mg of crystalline chymotrypsin. Acidification of the kidney extract, according to Leloir et al. ([10]), eliminates its capacity to destroy both substances.

These experiments suggest that the plasma and tissue peptidases which act on hypertensine are the same as those that destroy bradykinin and substance "V".

Paper Chromatography. We tried to find out whether substance "V" and

(*) Armour Laboratories.

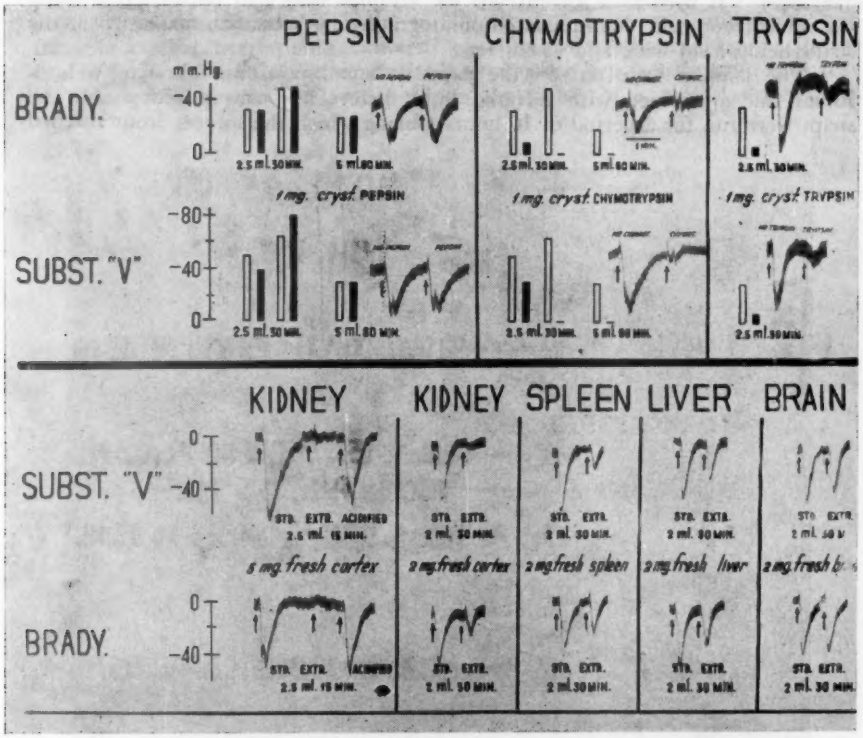

FIG. 7.—*(Above) LEG PERFUSION PRESSURE. INFLUENCE OF ENZYMES ON BRADYKININ AND SUBSTANCE V.*

Pepsin: *Two concentrations of crystalline pepsin were used: 1 mg in 2,5 ml final volume (columns to the left) and 1 mg in 5 ml final volume (columns to the right). Each pair of columns represents one experiment. White columns are the blanks: without the enzyme; black columns are incubations with the enzyme. In the first case, 5 U of bradykinin or substance V were incubated at pH 1 for 30 minutes. In the second case the incubation lasted 60 minutes (the concentrations of bradykinin and substance V being the same as in the first experiment).*

Chymotrypsin: *Same as above, but incubated at pH 7,4 (using phosphate buffer).*

Trypsin: *1 mg crystalline trypsin in 2,5 ml final volume incubated at pH 7,4 with 5 depressor units of substance V or bradykinin.*

Both substance are resistant to pepsin, but are destroyed by chymotrypsin and trypsin.

(Below) INFLUENCE OF ORGAN EXTRACTS ON SUBSTANCE V AND BRADYKININ. Organ extracts containing 100 mg of fresh tissue in 1 ml were incubated at pH 7,4 in the conditions shown in the figure, with 2 U of substance V or bradykinin. In the first kidney experiment the amount of both substances was 5 U and the incubation time 15 minutes, showing that the kidney extract previously acidified had lost its power to destroy both substances.

The sequence of destroying ability appears to be kidney-spleen-liver-brain.

bradykinin could be differentiated by this method. Butanol: water, acetic acid (25:25:6) was used as a solvent; all the samples were run by duplicate using the same trough, in descendent chromatography in Whatman paper Nº 3, the strips being 3 cm wide and 45 cm long.

The samples were put on the paper in small portions, following a horizontal line and dried with infrared light, before any new addition. All the strips were run for a period of 16 hours, during which the solvent front reached

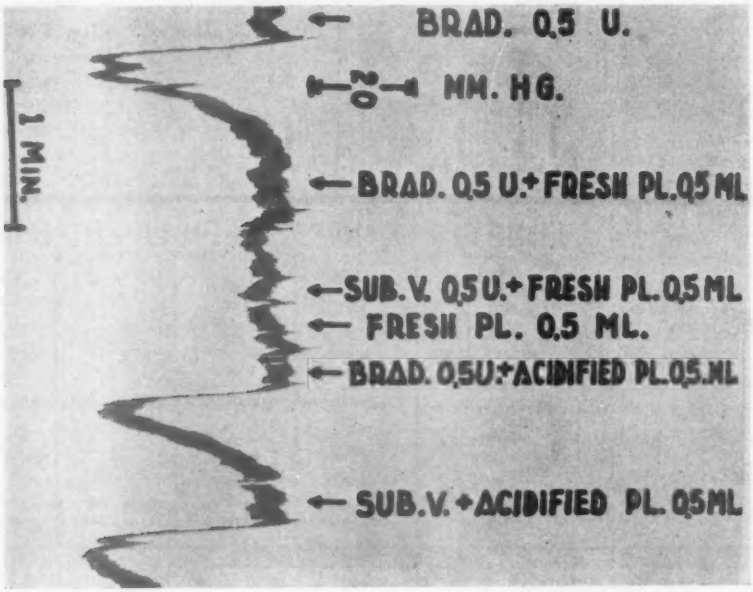
BRAD. 0,5 U.
20
MM. HG.
1 MIN.
BRAD. 0,5 U.+ FRESH PL. 0,5 ML
SUB. V. 0,5 U.+ FRESH PL. 0,5 ML
FRESH PL. 0,5 ML.
BRAD. 0,5U.+ACIDIFIED PL. 0,5 ML
SUB. V. + ACIDIFIED PL. 0,5 ML

FIG. 8.—*EFFECT OF FRESH AND ACIDIFIED DOG PLASMA ON BRADYKININ AND SUBSTANCE V. 2 U bradykinin or 2 U substance V were incubated with 2 ml fresh plasma and with plasma previously acidified to pH 4, during 15 minutes at 37° C. Equal amounts (0.5 ml) of bradykinin without plasma, bradykinin and substance V incubated with fresh plasma, a "blank" of fresh plasma, and both substances incubated with acidified plasma, were injected in the perfused leg.*

28 - 30 cm. The strips were dried, cut into pieces 1 or 2 cm wide, and eluted with 2 ml of distilled water or Ringer solution. Until the moment of the biological test they were kept in a freezer at —20° C, bradykinin and substance "V" were recognized by their depressor effect on the perfused leg; hypertensin by its pressor effect on the anesthetized rat.

Both bradykinin and substance "V" Rf. values appear to be strongly influenced by the period of time elapsed after the mixture of the solvent components, possibly due to the formation of butyl acetate. In experiments with fresh solvent (24 hours after mixture) the Rf. for hypertensin was 0.13 and 0.18 for substance "V" (using hypertensin preparation PC 2, prepared as PC 1). When the solvent was a week or more old we found in several experiments

Rf. values of 0.98, 0.94 and 0.82 for substance "V", and 0.83, 0.94 and 0.70 for bradykinin, taking into consideration the most active portion of the strip. The zone covered by the activity has a variable length, between 4 and 6 cms, and there is a great overlapping between substance "V" and bradykinin. In the case of preparation PC 2, we were unable to separate hypertensin from substance "V" using this method. Figure 9 shows the result of a typical experiment.

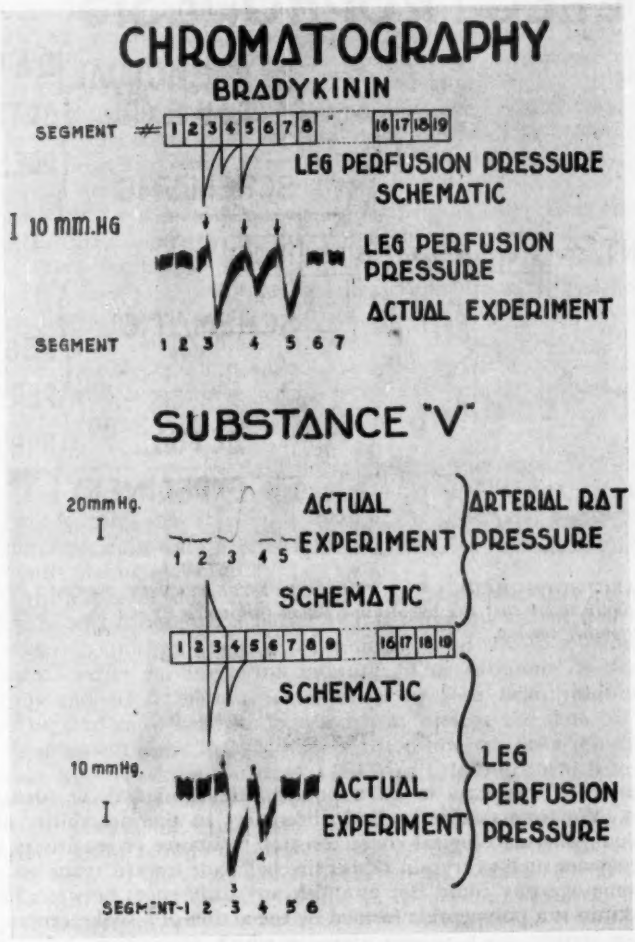

FIG. 9. — *CHROMATOGRAPHY OF BRADYKININ AND SUBSTANCE V. The figure shows that bradykinin is found in the same zone of the paper strip as substance V and hypertensin both contained in the preparation PC1.*

Electrophoresis. We tried to separate hypertensin from substance "V" in preparation PC 1, by the electrophoretic method. Whatman paper Nº 1, and Walpole acetate buffer pH 4, ionic strength 0.05, were used. Electric current was set at 220 volts during 7-8 hours. In all the experiments, hypertensin and substance "V" were found in the same zone and occasionally the maximun activity was in different but contiguous segments of the strip (fig. 10).

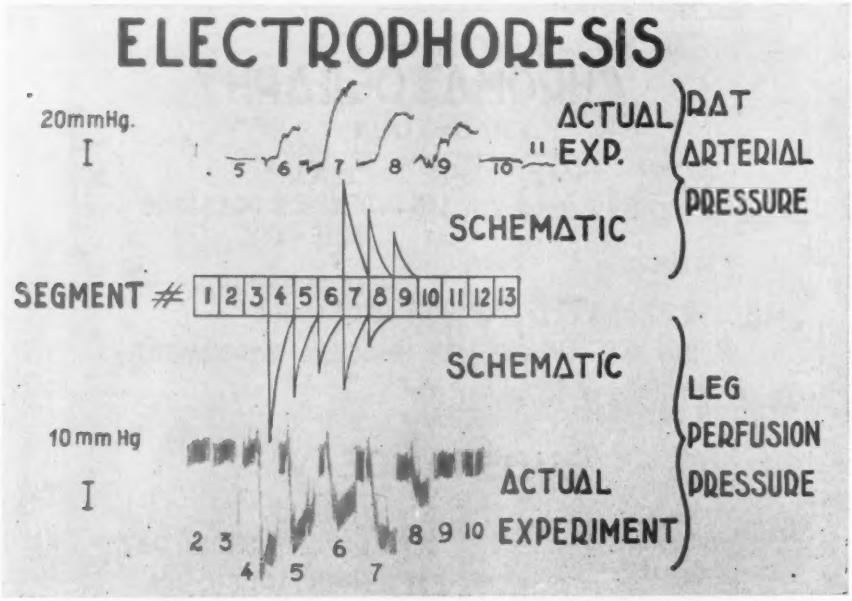

FIG. 10.—*ELECTROPHORESIS OF A PURIFIED HYPERTENSIN PREPARATION (PCl). The figure shows that the hypotensive action (substance V) and the hypertensive action (hypertensin) overlap.*

DISCUSSION

Substance "V" appears to be a polypeptide similar to or identical with bradykinin. We were unable to find differences in thermostability in acid or basic medium, pharmacological characteristics, resistance or sensitivity to several digestive enzymes such as trypsin or pepsin, behavior toward tissue enzymes, etc. Paper chromatography could not establish any differences between them.

Bradykinin is a polypeptide formed by the action of a snake venom (botrops jararaca) or trypsin, on a plasma globulin which precipitates between 35 and 45 % saturation with ammonium sulphate. It is a smooth muscle stimulating substance and it produces a fall in the blood pressure when injected intravenously. Bradykinin cannot at present be differentiated chemically or pharma-

cologically from kallidin, a substance formed by the incubation of kallikrein with a plasma precursor present in the globulin fraction. Kallikrein is a protein present in large quantities in pancreatic juice, in salivary glands and in saliva, and in smaller quantities in the kidneys ([22]).

The different kallikrein preparations are not identical. Pancreatic and urinary preparations are chemically and pharmacologically different from the submaxillary substance. The kallidins produced by them all seem to be identical.

Substance "P", a potent smooth muscle stimulating factor extracted from intestine is not identical with bradykinin. The two polypeptides can be separated by counter-current methods and paper electrophoresis ([14]). Furthermore, Zetler ([23]) found that substance "P" has several effects on the central nervous sysem of the rat, which are not produced by bradykinin.

Darmstoff, a smooth muscle stimulating substance isolated from the small intestine was once believed to be identical with substance "P". Nevertheless at present it is known to consist of several acidic phospholipids, one of which apears to be an acetalphosphatidic acid ([21]).

Substance "U" is a smooth muscle stimulating and depressor substance of polypeptide nature, found by Beraldo ([1]) to be present in dog urine. This substance is very similar to bradykinin and is possibly the same substance.

Clostridin is a substance formed by the action of a cysteine-activated protease secreted by clostridium histolyticum on a globulin fraction of bovine plasma. Clostridin is similar to bradykinin if not identical with it ([15]).

Kinin ([18]), a polypeptide present in wasp venom, is a potent smooth muscle stimulating and hypotensive agent. Despite its many common properties, kinin could be distinguished from kallidin or bradykinin by paper chromatography.

The present data support the conclusion that substance "V", bradykinin, kallidin, substance "U" and clostridin are identical or very closely related polypeptides. It seems that trypsin, kallikrein, the venoms of bothrops jararaca, and clostridium histolyticum give rise to the same depressor polypeptide when incubated with plasma globulins.

The significance of substance "V" in such great quantities in our hypertensin preparations is obscure. Schacter ([19]) has recently shown that the simple dilution of serum (ox, guinea pig, rat, human) give rise to a "slow contracting substance" active on uterus or intestine. This substance is destroyed by chymotripsin and its formation is inhibited by soya bean inhibitor and it appears to be similar to bradykinin, though at present the data do not allow to draw final conclussions in this respect. According to Schacter the dilution of the serum will transform kallikreinogen into kallikrein, which acting upon the serum globulins will form kallidin.

When the plasma is treated with chloroform or brought into contact with a glass surface, kinin activity develops; this is probably due to the activation of plasminogen to form plasmin, which as shown by Beraldo ([2]) acting on the plasma protein forms bradykinin. In fact, in this case antiplasmin inhibits the formation of bradykinin ([12]).

Recently Lewis and Work ([11]) confirmed Beraldo's findings, showing that the incubation of human plasmin with a pseudoglobulin fraction of human plasma originated a "slow contracting substance", resistent to atropine and presumably similar to the bradykinin group. The activation of plasminogen

can result from the action of 5-hydroxytryptamine, recently shown to be a potent agent in stimulating the production of fibrinolytic activity (9).

It seems possible that substance "V" could be formed in the ox plasma used for preparing hypertensinogen, before precipitating it with ammonium sulphate, or later. It could also be formed by the action of some renal enzyme (kallikrein?) contained in our crude renin preparation, on the plasma globulins.

Summary and conclusions

A vasodilator substance (substance V) present in a purified hypertensin preparation is described.

Substance V produces strong vasodilatation when injected intraarterially (or in the blood leading to a perfused organ) but has a much weaker effect if the injection is given intravenously. This is due to the fact that substance V is rapidly inactivated by blood plasma.

Substance V is not inactivated by boiling 20 minutes in an acid medium, but most of the activity is lost by a similar treatment in 0.1 N NaOH. It is dialysable through cellophane membranes.

Substance V is destroyed by chymotrypsin but not by pepsin. Blood plasma and several organ extracts rapidly inactivate substance V. This action is lost if the plasma or the extracts are previously acidified to pH 4 and kept at 37° C for 30 minutes.

Substance V appears to be a polypeptide similar to or identical with bradykinin. Both substances have been compared and found to have the same properties in the characteristics listed above. Furthermore they could not be differentiated by paper chromatography.

References

(1) Beraldo, W.: *Amer. J. Physiol.*, 1952, *171*, 371.
(2) Beraldo, W.: *Amer. J. Physiol.*, 1950, *163*, 2.
(3) Braun Menéndez, E., Fasciolo, J. C., Leloir, L. F., Muñoz, J. M., Taquini, A. C.: *Hypertensión arterial nefrógena.* Edit. El Ateneo, Buenos Aires, 1943.
(4) Calvo, R., Fasciolo, J. C., Chionetti, J. I., Suárez, J. R.: *Rev. argent. Cardiol.*, 1956, *137*, 229.
(5) Euler, U. S., Gaddum, J. H.: *J. Physiol.*, 1931, *72*, 74.
(6) Fasciolo, J. C., Leloir, L. F., Muñoz, J. M., Braun Menéndez, E.: *Rev. Soc. argent. Biol.*, 1940, *16*, 43.
(7) Fasciolo, J. C.: *Acta physiol. latinoamer.*, 1953, *3*, 1.
(8) Frey, E. K., Kraut, H., Werle, E.: *Kallikrein.* Edit. Enke, Stuttgart, 1950.
(9) Kwaan, H. C., Lo, R., McFadzean, A. J. S.: *Clin. Sc.*, 1957, *16*, 255.
(10) Leloir, L. F., Muñoz, J. M., Braun Menéndez, E., Fasciolo, J. C.: *Rev. Soc. argent. Biol.*, 1940, *16*, 635.
(11) Lewis, G. P., Work, T.: *J. Physiol.*, 1957, *135*, 7P.
(12) Lewis, G. P.: *J. Physiol.* 1958, *140*, 285.
(13) Paladini, A. C., Braun Menéndez, E.: *Biochim Biophys. Acta*, 1955, *18*, 580.
(14) Pernow, B., Rocha e Silva, M.: *Acta phys. scandinav.*, 1955, *34*, 59.
(15) Prado, J. L., Monier, R., Prado, E. S., Fromageot, C.: *Biochim. Biophys. Acta*, 1956, *22*, 87.
(16) Rocha e Silva, M., Beraldo, W., Rosenfeld, G.: *Amer. J. Physiol.*, 1949, *156*, 261.
(17) Rocha e Silva, M.: *Arch. internat. pharmacodyn.*, 1951, *8*, 271.
(18) Schacter, M., Thain, E. M.: *Brit J. Pharmacol.*, 1954, *9*, 352.
(19) Schacter, M.: *Brit. J. Pharmacol.*, 1956, *11*, 111.
(20) Vogt, W.: *Arch. exper. Path. u. Pharmakol.*, 1949, *206*, 1.
(21) Vogt, W.: *J. Physiol.*, 1957, *137*, 154.
(22) Werle, E., Maier, L.: *Biochem. Ztschr.*, 1952, *323*, 279.
(23) Zetler, G.: *Arch. exper. Path, u. Pharmacol.*, 1956, *229*, 148.

STRESS AND THE ELECTROLYTE-STEROID-CARDIOPATHY (ESCN)

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IT HAS BEEN the major endeavor of our Institute to prove that hormones can play a decisive role in the development of numerous non-endocrine diseases, that is, of maladies which are not primarily due to derangements originating in the endocrine glands themselves. Most of our work has been concerned with the participation of hormones in the pathogenesis of inflammatory diseases, nephrosclerosis and hypersensitivity reactions. The major results of these investigations have recently been summarized in book form [19], so that they need not be considered here.

During the last year, however, it became evident that, corticoids can also condition the cardiac muscle for the production of structural changes by electrolytes. It was observed, that *infarct-like, massive patches of cardiac necrosis can regularly be produced by combined treatment with certain sodium salts (e.g., Na_2HPO_4, NaH_2PO_4, $NaSO_4$, $NaC10_4$) and various corticoids,* although such changes do not develop when either the electrolytes or the steroids are given alone [20, 36]. On the other hand, simultaneous administration of $MgCl_2$ or KCl was found to be effective in protecting the heart against this kind of damage [28, 37]. Our studies on the "Electrolyte-Steroid-Cardiopathy," characterized by necrosis of the myocardium, have been the subject of an extensive monograph [30] which served as a basis for this review. It was felt that these newly discovered interactions between electrolytes and steroids may help to elucidate some fundamental problems of cardiac physiology, including the biochemical basis of those cardiac infarctions not due to demonstrable, acute vascular obstructions.

From the clinical point of view, perhaps, the most important finding is that in the phosphate plus corticoid-conditioned rat, even mere exposure to non-specific stressors (neuromuscular exertion, traumatic shock, vagotomy, etc.) can elicit focal myocardial necroses with inflammation [36, 40]. This type of cardiac damage, as well as a number of other experimental cardiopathies (e.g., the myocardial necroses and inflammations caused by the intravenous injection

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of proteolytic enzymes or by combined treatment with vitamin-D derivatives and NaH_2PO_4), could also be prevented by the prophylactic administration of $MgCl_2$ or KCl ([31, 35]). It gradually became evident that electrolytes, steroids and stress all play important conditioning roles in the development of cardiopathies. Therefore, further studies that are likely to develop our knowledge in these fields are of special importance, because it is hoped that they may supply fundamental data for the understanding and prevention of cardiopathies, not only in experimental animals, but also in man.

This communication deals with experiments that demonstrate the ability of numerous stressors and very diverse agents to elicit manifestations of infarctoid cardiopathy, in rats specifically "conditioned" for this unspecific type of response by treatment with 2∝-methyl-9∝-chlorocortisol. But before proceeding with the description of our recent observations, it will be necessary to give a brief synopsis of the scattered and polyglot literature concerning the pathogenesis and clinical implications of the electrolyte-steroid-cardiopathy and other infarctoid cardiopathies.

The "electrolyte-steroid-cardiopathy"

Historical data. It has been well known for half a century that focal myocardial necroses, followed by inflammation, can occur in man, either as an apparently separate disease (Fiedler's myocarditis) or as part of systemic infections (e.g., in diptheria, typhus). A review of the literature has shown, furthermore, that cardiac glycosides ([9]), vitamin-D derivatives ([14]), adrenaline ([5, 6]), potassium deficiency ([13]), forced muscular exercise ([3])—i.e., a number of apparently quite unrelated agents—can elicit essentially similar lesions in the myocardium of experimental animals. The principal structural characteristics (necrosis, calcification, inflammation) of these lesions, as well as their extent, position and speed of development, vary somewhat from case to case, but they have certain salient common features: 1) morphologically, they are characterized by focal necrosis with invasion of the damaged muscle tissue by inflammatory cells; 2) unlike the true cardiac infarcts, this necrosis is not due to acute vascular obstruction, but presumably to biochemical changes in the myocardium. None of the earlier authors stressed these similarities. Fiedler's myocarditis, the pharmacology of digitalis, the effects of adrenaline overdosage, the toxicology of vitamin-D derivatives, the cardiovascular effects of muscular work and the essentiality of potassium as a nutrient are topics so far removed from each other that no investigator interested in any one of them could be expected to be well informed about the literature in all these fields.

According to the concept of the "diseases of adaptation," an excess of corticoids could be expected to produce morbid changes in organs that are particularly affected by systemic stress ([17]). Hence, as soon as adequate amounts of pure synthetic corticoids became available, we wanted to determine whether structural lesions would be produced in the cardiovascular system by such hormones. As a result of these investigations, in 1940 we noted that chronic treatment with desoxycorticosterone produces marked cardiac and renal hypertrophy in the rat ([16]). However, there appeared to be no very specific pathologic changes in these enlarged hearts. After numerous experiments in 1943 we finally succeeded in producing fatal cardiovascular damage with desoxycorti-

costerone, with typical hyalinizing myocarditis, periarteritis nodosa, nephrosclerosis and hypertension [34]. This was the first experimentally produced *E*lectrolyte plus *S*teroid-induced *C*ardiopathy characterized by *H*yalinization, the type of change to which we now refer as the *"ESCH"*. This hyalinizing cardiopathy resembles certain types of rheumatic carditis, and the extracardiac lesions (periarteritis nodosa, nephrosclerosis and malignant hypertension), which accompany this change in the desoxycorticosterone-treated rats, also have their counterparts in clinical pathology. In 1957, the discovery of the *E*lectrolyte-*S*teroid *C*ardiopathy with *N*ecroses (instead of hyalinization), the *"ESCN"*, greatly helped to clarify the fundamental difference between the hyalinizing and the necrotizing types of cardiopathy. It became evident that, under comparable conditions, the same steroids can predispose the rat for the production of either kind of cardiac lesion, but different electrolytes are necessary to elicit one or the other type of change.

The steroid component in the "ESCN". – The ESCN was first observed in rats conditioned by injections of a highly active synthetic corticoid, 2α-methyl-9α-chlorocortisol (Me-Cl-COL), and simultaneously given an excess of dietary phosphate. On the other hand, even fatal doses of the steroid or the phosphate alone failed to produce myocardial necroses [20, 36, 38]. As Me-Cl-COL exerts both, mineralo-and glucocorticoid properties, it was our next task to establish whether the cardiotoxic effect is more closely related to the mineralocorticoid or to the glucocorticoid action; it is possible that both types of activity are important. Among 48 steroids that have been tested as regards their cardiotoxicity so far, Me-Cl-COL proved to be most effective in producing the *"ESCN"*. However, 2α-methyl-9α-fluorocortisol and the two corresponding, non-methylated steroids (9α-chlorocortisol and 9α-fluorocortisol) are likewise very effective in this respect. Among those examined, the only halogenated corticoid devoid of this activity is triamcinolone (Δ[1]-9α-fluoro-16-hydroxycortisol), a highly active glucocorticoid which possesses little or no mineralocorticoid potency. On the other hand, cortisone, a glucocorticoid with little mineralocorticoid activity, is at least as effective in producing "ESCN" as is desoxycorticosterone, although the latter is much more mineralocorticoid than is the former [39]. It is especially noteworthy that, when given in combination with NaH_2PO_4, even cortisol, the principal natural glucocorticoid of man, can produce such cardiac changes; the same is true of the natural corticoid mixture that is secreted by the adrenals of animals treated with large amounts of ACTH [22, 39].

All the steroid hormones that we found to be highly effective in producing the "ESCN" are potent corticoids. In experiments in which neither high doses of triamcinolone (a pure glucocorticoid) nor of desoxycorticosterone (a pure mineralocorticoid) produced any marked degree of cardiopathy in NaH_2PO_4 sensitized rats, combined treatment with small doses of both these steroids sufficed to induce pronounced and extensive cardiac necroses [23]. It would appear, therefore, that the concurrent administration of mineralo-and glucocorticoids is most effective in conditioning the cardiac muscle to the necrotizing action of certain electrolytes. This finding seems to be especially interesting, because in so many other respects these two types of corticoids are antagonists. Under our experimental conditions, none of these steroids produced cardiac necroses when they were not supplemented by treatment with electrolytes.

The electrolyte component in the "ESCN". As mentioned before, the "ESCN" was accidently discovered in rats injected with Me-Cl-COL and treated simultaneously with large amounts of NaH_2PO_4 by stomach tube ([20, 36]). Soon after this observation, we learned that both NaH_2PO_4 and Na_2HPO_4 (as well as neutral mixtures of both these salts) are highly active in this respect. It was assumed, therefore, that this infarctoid cardiopathy is not due merely to changes in pH that might be induced either by the acid or by the basic phosphate ([38]). When NaH_2PO_4 and NaCl were given in equimolecular amounts to rats conditioned with Me-Cl-COL, only the former salt caused necroses in the myocardium. The inactivity of NaCl led us to conclude at the time that, for the production of the infarctoid cardiopathy, the phosphate ion is the essential part of NaH_2PO_4. This is the reason why, in our first papers, the "ESCN" was described as a "phosphate-steroid-cardiopathy". But it will be seen that the assumption that PO_4 is the active moiety in the sodium phosphate was not entirely correct, because Na is likewise indispensable.

Subsequently, it has also been possible to produce similar cardiac lesions by combined treatment with Me-Cl-COL and other inorganic phosphorus compounds, such as: NaH_2PO_3, Na_2HPO_3, NaH_2PO_2, $Na_4P_2O_7$ and $(NaPO_3)_6$ ([24]). We then learned that not only phosphates and their immediate derivatives but many other Na-salts can produce cardiac necroses under these experimental conditions. Na-sulfates and sulfites, as well as $NaClO_4$, are approximately as active as the corresponding phosphorus compounds ([28, 29]). As the chloride and several other organic and inorganic sodium salts were found to be ineffective, the question arose whether the cation plays any role in the production of the ESCN. KH_2PO_4 (unlike NaH_2PO_4) does not produce an ESCN in the Me-Cl-COL conditioned rat ([10]). None of 18 non-sodium salts tested elicited the typical picture of the FSCN. This is all the more noteworthy because we purposely employed phosphates, sulfates and perchlorates, that is, salts of anions that are highly effective in activating the ESCN-producing effect of Na ([30]).

Our general conclusions from all these observations can be summarized as follows: 1) only Na-salts are active in producing an ESCN; 2) not all Na-salts permit the production of an ESCN; 3) both the cation and the anion play important roles in the production of the ESCN; 4) Na occupies a central position and certain anions, especially PO_4, SO_4 and ClO_4, sensitize the heart for the cardiotoxic actions of this cation.

After these facts were established, the question arose whether anions that do not sensitize (for example, Cl) are merely inert or whether they actively inhibit the efficacy of Na. Systematic studies were performed, therefore, on rats treated with a series of Na-salts in addition to Me-Cl-COL plus Na_2HPO_4. Among all the sodium salts tested, only NaCl exerted a statistically significant protective action ([30,33]). This inhibition by one Na-salt of cardiac necroses produced by other Na-salt offers the most convincing proof of the active role played by anions.

It has been briefly mentioned in the introduction, that $MgCl_2$ and KCl inhibit the morbid changes that are produced in Me-Cl-COL conditioned rats by simultaneous treatment with certain Na-salts (particularly the phosphates, sulfates and perchlorate). However, in this respect, under standard experimental conditions, equivalent amounts of $MgCl_2$ or KCl are far more effective than is NaCl ([23, 28, 37]). Comparative experiments with other electrolytes clearly

showed that the cation is likewise of importance, since certain chlorides proved to be far more effective than others in preventing the ESCN. In particular, $MgCl_2$ and KCl were most active, NH_4Cl and $CaCl_2$ exhibited a moderate anti-ESCN activity, while NaCl was least effective. The nature of this desensitizing action is still obscure, but since even the forced oral administration of water gave some protection against the ESCN, the possibility had to be considered that both water and the chlorides might act merely by increasing the urinary excretion of toxic electrolytes ([33]). It has also been definitely proven that the phenomenon of desensitization does not depend upon interference with any specific effect of the phosphate-ion, since the protective effect on $MgCl_2$ or KCl is also manifest in Me-Cl-COL conditioned rats in which ESCN is produced by Na_2SO_4 or $NaClO_4$ instead of by Na_2HPO_4 ([28, 30]). To further our study of the important phenomenon of protection, a great number of electrolytes were tested, in various combinations. All these investigations suggested the following general conclusions:

1) The production of an ESCN by sensitizing Na-salts can be prevented by a variety of chlorides; 2) KCl and $MgCl_2$ are the most active among the relatively nontoxic chlorides that we have tested; 3) a number of K-salts other than the chlorides likewise exhibit considerable anti-ESCN activity, while the corresponding Mg-salts are less active or actually inert; 4) the anti-ESCN action of various K-salts, other than the chloride, is greater than that of Mg, but KCl and $MgCl_2$ are about equally active; 5) both the cation and anion play important roles in the prevention of the ESCN: 6) there exists a strictly quantitative relationship between sensitizing and desensitizing electrolytes in that ¼ to ½ mM of KCl or $MgCl_2$ is the threshold amount necessary to produce a just significant inhibition of an ESCN induced by 1 mM of Na_2HPO_4 (for literature see Ref. [30]).

The possibility had to be considered that sensitizing and desensitizing electrolytes might inactivate each other, owing to chemical reactions that take place immediately after these salts are introduced into the gastrointestinal tract. It has been found, however, that the oral administration of desensitizing salts counteracts the effect of sensitizing electrolytes, even if the latter are injected parenterally ([23]).

The extracardiac effects of electrolyte steroid treatment are not our principal concern here; yet, it is worth mentioning that the production by Me-Cl-COL plus sensitizing electrolytes of lesions in the heart, kidney and liver does not run parallel. Only phosphates and their close derivatives produce significant amounts of nephrocalcinosis, while hepatic necrosis is induced by a larger variety of salts and particularly by the Na-sulfates, Na_2SO_3, NaH_2PO_3, $NaClO_4$ and Na-tartrate ([30]).

Blood pressure in the "ESCN". – It has been shown that various electrolytes influence the pressor activity of corticoids. On the other hand, the desoxycorticosterone-hypertension, as well as the associated ESCH (with periarteritis and nephrocalcinosis), is aggravated by Na-salts ([17]). It was, therefore, necessary to establish the relationships between ESCN and blood pressure. In rats treated with NaH_2PO_4 plus Me-Cl-COL, there was a slight initial hypertension not significantly greater than that induced by Me-Cl-COL alone. NaH_2PO_4, in itself, did not alter the blood pressure in these experiments. However, in the animals

that received the combined electrolyte steroid treatment, the blood pressure fell below normal, several days before death from myocardial necroses. Here, the ante-mortem hypotension was presumably due to heart failure induced by the myocardial necroses. There is no evidence that the ESCN is preceded by any pronounced hypertensive episodes ([1]).

ECG in the "ESCN". — The ECG was registered—using the three standard leads—in rats treated with Me-Cl-COL and NaH_2PO_4 under the usual conditions of experimentation for the induction of the ESCN. The most conspicuous changes observed were: a decrease of the heart rate with prolongation of the PR and QT (QU) intervals and a lowering of the T waves. All these ECG-anomalies became evident as early as two days after initiation of the treatment—that is, long before the appearance of the massive cardiac necroses. However, after seven days, when large patches of ESCN appeared, the ECG began to reveal arrhythmias and conduction defects ([42]).

Biochemical data. — The distribution of Na, K and inorganic PO_4 was studied in the serum, abdominal muscles and heart of the rat, in relation to the ESCN. The K-concentration was decreased in the heart and skeletal muscles of rats treated with NaH_2PO_4 and/or Me-Cl-COL. A significant increase in the Na-concentration of the heart was even more marked after combined treatment with both these agents. On the other hand, there was no significant change in the organic PO_4-content of the heart during any of these treatments ([4]).

Histologic characteristics of the "ESCN". — The lesions of the ESCN resemble infarcts but are not identical with them. Like the true cardiac infarcts, the infarctoid foci consist of massive, necrotic patches within whose limits the muscle fibers are gradually absorbed and replaced, first by histiocytes and polymorphonuclear cells, later by connective tissue. Any part of the heart may be affected, but the right ventricle, the auricles and the subendocardial layers of both ventricles are sites of predilection. The necrosis appears to be the result of the primary change in the myocardial fibers themselves, since these show the earliest lesions. It is in this respect that the ESCN produced by corticoids and electrolytes differs most markedly from the classical cardiac infarct that results from acute obstruction in the coronary vessels.

With the aid of special stains which we have recently perfected, it was possible to demonstrate the accumulation of a strongly fuchsinophilic material which eventually displaces all other elements within the cardiac muscle fibers, and results in necrosis. This change usually begins in the immediate vicinity of nuclei and then tends to spread, so that ever larger portions of a muscle fiber are transformed into a more or less hyalin, strongly fuchsinophilic mass. At this stage, the rest of the muscle fiber, including its nucleus, may still retain a histologically normal appearance. As the lesion progresses further, the nucleus becomes indistinct and eventually disappears, while an entire segment of the muscle fiber, still sharply delimited by sarcolemma and intercalated discs, is completely transformed into a hyaline, fuchsinophilic tube. When only individual fibers or fiber segments are affected by this change, the lesion heals without causing inflammation or leaving a permanent scar. However, when many adjacent fibers are thus affected and the lesion assumes a "critical size". healing is possible only after the damaged tissue debris is removed by inflam-

matory cells and replaced by a connective-tissue scar. The fuchsinophilic material apepars to be characteristic only of the initial stages of cardiac damage; it disappears completely from muscle fibers that have totally disintegrated and are in the process of being absorbed.

Among other degenerative changes that tend to occur in the early stages of the ESCN, extensive calcium deposition in the affected myocardial fibers is the most common, granules of calcium salts (tingible with von Kossa's method) are often detectable, even in muscle fibers which still retain their cross striation and whose nucleus does not exhibit any manifest abnormality. It would appear, therefore, that calcium deposition is not necessarily the consequence of necrosis, although it occurs most commonly in necrobiotic or frankly necrotic fibers ([30, 36]).

"ESCN" and coronary sclerosis. — We have already seen that, in the ESCN, myocardial necroses occur without any morphologic evidence of a vascular obstruction. It must be kept in mind, however, that the development of all cardiac infarcts depends primarily upon the relationship between the metabolic requirements and the blood supply, of the heart. Hence, combined treatment with active Na-salts and corticoids might act by inducing potentially dangerous metabolic changes within the myocardium, so that even the physiologic, normally well tolerated, variations in its blood supply may result in necrosis. In this case, the precipitating effect of stress could be due either to an additional increase in the metabolic demands of the cardiac muscle, or to temporary variations in its blood supply that are not reflected in any detectable structural change within the coronary vessels. If this concept is correct, the ESCN may help to clarify the pathogenesis of those cardiac infarcts of man which are unaccompanied by acute vascular obstruction.

All our experiments concerning the ESCN were performed on healthy young animals with normal cardiac vessels, while spontaneous infarcts tend to occur with the greatest frequency in middle aged or older men, in whom even a moderate degree of coronary sclerosis may well act as a predisposing factor for metabolic changes similar to those induced by electrolytes plus corticoids. We thought that in order to elucidate this problem it would be useful, first to produce a moderate amoutn of coronary sclerosis and hardening (for instance, by treatment with dihydrotachysterol, "DHT") and then, to determine whether such an "artificial aging" of the arterial system can sensitize the heart to the necrotizing effect of subsequent treatment with electrolytes and corticoids. Therefore, albino rats were pretreated with DHT, during 10 days, so as to produce mild narrowing and calcification of the coronary arteries. After this, no further treatment was given until all the animals had recovered, as judged by their growth and clinical appearance. Then, combined treatment with NaH_2PO_4 plus Me-Cl-COL was initiated at a dose level which, in non-pretreated animals, caused only occasional microscopic foci of myocardial necrosis, localized in the areas of predilection (the atria, the right ventricle and the subendocardial layers of both ventricles). It was found that, in the DHT-pretreated rat this dose level of NaH_2PO_4 plus Me-Cl-COL produces particularly extensive, macroscopically visible patches of necrosis throughout the heart. This finding appeared to support our hypothesis, but quite unexpectedly it was also noted, after sensitization with DHT, intense proliferative and eventually obstructive changes develop in all parts of the coronary tree during NaH_2PO_4 plus Me-Cl-COL treatment ([25]). The mechanism of this vascular sensitization is not yet clear,

but these observations suggest that the electrolyte-steroid effect is not necessarily limited to the cardiac muscle itself, but may also participate in the production of obstructive coronary lesions.

The question of species-specificity. – The medical implications of all these observations are largely dependent upon the demonstration that the production of such cardiac necroses is not merely a species-specific reaction type, peculiar to the rat. It is significant, therefore, that similar cardiac lesions have also been produced by Me-Cl-COL and NaH_2PO_4 in the guinea-pig, hamster, rabbit and dog ([11, 12]). Since the principal glucocorticoid of man is cortisol, it is especially noteworthy that extensive myocardial necroses can likewise be produced in primates (rhesus monkeys) by combined treatment with cortisol and NaH_2PO_4 ([41]). It is probable, therefore, that the cardiac muscle of man is also sensitive to this type of action.

Other infarctoid cardiopathies

From a review of the literature, the following conclusions can be drawn: many drugs and food constituents can produce focal myocardial necroses, at least under certain experimental conditions (for literature see Ref.30). Virtually nothing is known about the mechanism through which these latter agents affect the cardiac muscle, but preliminary studies have convinced us that the production of cardiac necroses by almost any means is likely to be influenced –at least to some extent–by those factors (steroids, electrolytes, stress) that determine the development of the typical ESCN.

Almost 30 years ago, it was noted that, in rats, certain steroids of the vitamin-D group can produce cardiac necroses with calcification ([15]). The question then arose whether this type of change could also be influenced by excessive dietary phosphate. Normally, DHT produces calcification and necrosis of the cardiac muscle but no inflammatory reaction; however, simultaneous treatment with NaH_2PO_4 transforms the character of these lesions, so that a suppurative, acute myocarditis results ([21]). Like the infarctoid cardiopathy, this inflammatory lesion can be prevented by the concurrent administration of $MgCl_2$ or KCl ([27, 35]).

Using large doses of arsphenamine in rats, we succeeded in producing typical focal myocardial necroses without any complicating skin lesions. It is evident, therefore, that the arsenicals themselves can induce myocardial changes of this type. Our preliminary experiments suggest, furthermore, that the cardiac lesions produced by threshold doses of arsphenamine can be aggravated by Me-Cl-COL or Na_2HPO_4 and inhibited by KCl_2 ([32]).

Previous investigators reported that a single intravenous injection of a proteolytic enzyme preparation (e.g., papain, streptokinase) produces histologic, as well as sometimes also macroscopically visible, lesions of the heart muscle ([7, 8]). We therefore performed systematic studies with papain, on rats under our usual experimental conditions for the production and prevention of the ESCN. This work showed that Me-Cl-COL and/or Na_2HPO_4 greatly sensitizes the heart to the production of myocardial necrosis by a single, subsequent intravenous injection of papain extract. On the other hand, $MgCl_2$ and KCl are extremely effective in protecting the heart against this type of enzymatic damage. It is significant that the hepatic necroses and renal changes that also occur in rats, following massive intravenous injections of papain, are quite similarly influenced

by Me-Cl-COL, Na_2HPO_4, $MgCl_2$ and KCl (31). It is generally assumed—although perhaps not definitely proven—that proteolytic enzymes, such as papain, produce necroses by acting directly upon the tissue proteins. If this is so, the observations just described would imply that the humoral agents which we employed can even modify the susceptibility of tissues to proteolysis, *in vivo*. It has been noted, furthermore, that in rats exposed to the severe stress of prolonged restraint, papain produces particularly severe necroses (2). It is noteworthy that within the papain-affected areas of heart muscle, Anitschkow-myocytes and multinucleated giant cells are commonly observed; therefore, certain pathogenic relationships were suspected between these lesions and spontaneous collagen diseases, such as rheumatic fever and periarteritis nodosa.

All these observations suggested that there may be some common pathway in the mechanism through which various agents can produce, first necroses, then inflammation and finally fibrosis, in the heart muscle. The finding that $MgCl_2$ and KCl can protect against cardiopathies elicited with most diverse agents, is the most unexpected, and perhaps of greater general significance as regards the physiology and pathology of heart muscle, because it suggests that the protection afforded by Mg- and K- salts are rather nonpecific.

The stress component in cardiac necrosis

From our earlier work on stress and the diseases of adaptation, we concluded that "there begins to emerge a new and somewhat more complex pathology, in which the main objects of our studies are no longer individual 'pathogens', but rather, 'pathogenic situations'" (18). This concept has subsequently (19) helped us to come closer to the understanding of a variety of disease (e.g., gastroduodenal ulcers, hypertension, nephrosclerosis, periarteritis nodosa, rheumatoid diseases), in which the interminable search for single, specific pathogens has, of necessity, been fruitless. These maladies are not due to any one causative factor, but to pathogenic constellations that can be induced in various ways. It may be concluded from the findings summarized in this paper that this is also true of the necrotizing cardiopathies and of certain types of myocarditis. The investigations enumerated below support this assumption.

In this respect it is important to distinguish between the ESCN that is produced gradually by the persistent action of electrolytes and steroids, the "infarctoid cardiopathies" that are produced by direct effect of certain drugs, and the sudden eliciting effect of stress to which we made only cursory reference in the introductory paragraph. To clarify the specificity of this eliciting mechanism, rats were briefly treated with small amounts of Me-Cl-COL plus phosphate. Although this pretreatment produced little or no cardiac damage, subsequent exposure to the stress of a neuromuscular effort (induced by trapping the rats on a board) immediately elicited severe and extensive cardiac necroses in 100 % of the experimental animals (36). It might have been thought that neuromuscular exertion acts specifically (not merely through its stressor effect); but in rats similarly sensitized with threshold doses of Me-Cl-COL plus phosphate, a high incidence of massive myocardial necroses could also be elicited by bilateral vagotomy, hot or cold baths, multiple bone fractures, crushing of intestines, quadriplegia (subsequent to transection of motor nerves) or toxic doses of adrenaline (40). Even pretreatment with less active steroids (such as cortisol or desoxycorticosterone) in combination with NaH_2PO_4 sensitizes the rat (26) and rhesus monkey (41) to the production of myocardial necroses by

restraint. It is interesting, however, that in preparing the heart for the production of acute, infarct-like changes by subsequent exposure to a neuromuscular exertion, cortisol is again more effective than desoxycorticosterone. One of our latest experiments—which will be published elsewhere— revealed furthermore, that it is also regularly possible to elicit myocardial necroses by restraint, after sensitization with Me-Cl-COL alone (that is, without sensitization by electrolytes), and that $MgCl_2$ or KCl, if given during the restraint, protects the heart against this kind of damage ([32]). From a clinical point of view, the finding that the effects of direct cardiotoxic agents (such as, papain—a proteolytic enzyme, or plasmocid—an enzyme inhibitor) are also significantly aggravated by neuromuscular strain ([2]) may be important. On the other hand, the fact that sudden muscular exertion can precipitate cardiac infarcts, especially in predisposed middle- aged or older men, is too well known to deserve special attention here.

All these earlier investigations demonstrated that, under certain conditions, stress, corticoids and dietary electrolytes exert a necrotizing effect on the heart muscle and also influence the cardiotoxic effects of many, otherwise unrelated agents.

On the basis of these findings, we assumed that the nonspecific activity can be ascribed to the stressor action of the agents used, but this was not definitely proven. In most of these experiments, the rats were sensitized to the action of stressors with corticoids plus sodium phosphates. Although the necrosis-eliciting effect of various stressors in these experiments was significant, some of the control animals sometimes showed heart lesions which resulted from the Me-Cl-COL plus phosphate pretreatment alone. The possibility of precipitating infarct-like, myocardial lesions by sudden exposure to stressors is especially important, in view of its obvious clinical implications. Therefore, our next task was to establish the necrotizing ability of a great variety of noxious agents in the rat, conditioned for this non specific type of response by treatment with a highly active corticoid (Me-Cl-COL)—a steroid that never produces cardiac necroses when given alone.

Experimental material and techniques

Four hundred and eighty young female rats (Sprague-Dawley strain), with an average initial body weight of 100 g. (range 90-110 g.), were divided into 48 equal groups. Twenty-four groups received no treatment till the eighth day of the experiment and served as controls, to show the eventual myocardial—necrotizing effect of the various agents (or stressors) used. The remaining groups were treated from the beginning until the termination of the experiment with 2α-methyl-9α-chlorocortisol acetate (Me-Cl-COL) as described below; these showed the ability of corticoid (Me-Cl-COL) overdosage to condition for the cardiotoxic effect of most diverse stressors. After this preparatory period all groups (e.g., controls and Me-Cl-COL-pretreated groups) were treated with various agents, in doses verified (by preliminary experiments) to be definitely damaging to rats of this sex, strain and size. Some of the stressors used (e.g., Na-fluoroacetate, dinitrophenol, diphtherotoxin, muscular exercise) were selected particularly because of contradictory reports in the literature, concerning their ability to produce cardiac necroses when these agents are administered without conditioning. Others (for example, diisopropylfluorophosphate, cold baths, or *pseudomonas aeruginosa* infection) were not previously known to produce this type of cardiac damage.

All stressors were applied during 24 hours only, between the eighth and ninth days of the experiment; in addition, as diphtherotoxin is slow acting when given subcutaneously, it was also administered on the sixth day. Unless otherwise indicated, single interventions (e.g., spinal-cord transection) were performed at 4 P.M., on the eight day, while double interventions (e.g., hemorrhage, injections of drugs) took place at 4 P.M. on the eighth, and at 9 A.M. on the ninth, days. All drugs were injected subcutaneously. The stressors used were:

Cold bath (immersion in icy water at 1° C, during three minutes, twice);

Hot bath (immersion in water at 48° C, during five minutes, twice);

Muscular exercise (two and one-half hours in the morning and three hours in the afternoon, in a drum, 12 inches in diameter, revolving at 18 revolutions per minute);

Restraint (the animals were strapped to a board by adhesive tape, in the prone position, once during 17 hours);

Motor denervation of extremities (both cervical plexuses, as well as the femoral and sciatic nerves, were severed, like all other surgical operations, under ether anesthesia);

Spinal-cord transection (with a thermocautery, at the height of the seventh cervical vertebrae);

Vagotomy (transection of both vagi, at the height of the thyroid);

Crushing of intestines (stomach, ileum and cecum, each crushed three times with a hemostat);

Hemorrhage (removal of 3 ml of blood from the jugular vein, twice);

Adrenaline or Noradrenaline (200 μg, in 0.2 ml of oil, twice);

Vasopressin ("Pitressin," 10 I.U., in 1 ml of water, twice);

Pentamethylenetetrazol ("Metrazol," 6 mg, in 0.2 ml of water, twice);

Diisopropylfluorophosphate (0.6 mg in 2 ml of oil, by stomach tube, twice);

Na-iodoacetate (2.5 mg in 0.2 ml of water, twice);

Na-iodoacetate (2. mg in 0.2 ml of water, twice);

Dinitrophenol (0.6 mg in 0.2 ml of water, twice);

Picrotoxin (0.2 mg in 0.2 ml of water, twice);

Na-arsenite (0.6 mg in 0.2 ml of water, twice);

Na-arsenate (2.5 mg in 0.2 ml of water, twice);

Digitoxin (750 μg, in 0.2 ml of water, twice);

Diphtherotoxin (1:2.5 dilution in 0.9 % NaCl; 0.5 ml, once on sixth day, 1 ml once on eighth day);

Pseudomonas aeruginosa infection (1:100 suspension in 0.9 % NaCl (turbidity, 50) into a subcutaneously prepared air-pouch, once on sixth day).

Me-Cl-COL administered in the form of microcrystals of its acetate (Upjohn Company), in 0.2 ml of water, subcutaneously, 500 μg/day, to the Groups indicated in Table I.

The doses listed above were given to the Me-Cl-COL-treated groups. However, by the eighth day, under the influence of this potent corticoid the bodyweight of the rats had dropped from 100 to 80 g, while that of the controls without pretreatment had risen to 110-113 g; to compensate for this difference, the dose of the stressors had to be adjusted accordingly, in these controls. Hence, the amounts of all drugs given to the animals not pretreated with Me-Cl-COL were higher, by one half, than those given to the pretreated animals (see Table I). The hemorrhage was similarly adjusted, in that one-third more blood was removed in the controls. No such correction was deemed necessary as regards

TABLE I

Stress and the electrolyte-steroid-cardiopathy (ESCN)

Group	Treatment *	Total doses of the stressor agents	Final body weight (g.)	Cardiac necroses (Grade 0 to 3)	Mortality (%)
1	None	—	122 ± 2.8	0	0
2	None + Me-Cl-COL	—	83 ± 3.8	0	0
3	Cold bath	—	126 ± 2.0	0	40
4	Cold bath + Me-Cl-COL	—	86 ± 3.2	2.6 ± 0.23	0
5	Hot bath	—	122 ± 3.0	0	0
6	Hot bath + Me-Cl-COL	—	76 ± 3.8	1.3 ± 0.20	0
7	Muscular exercise	—	126 ±2.5	0	0
8	Muscular exercise + Me-Cl-COL	—	89 ± 3.4	1.7 ± 0.36	10
9	Restraint	—	112 ± 3.5	0.6 ± 0.20	0
10	Restraint + Me-Cl-COL	—	83 ± 2.0	2.8 ± 0.10	10
11	Motor denervation of extremities	—	110 ± 2.5	0	10
12	Motor denervation of extremities + Me-Cl-COL	—	89 ± 3.8	1.0 ± 0.27	10
13	Spinal-cord transection	—	115 ± 3.0	0	0
14	Spinal-cord transection + Me-Cl-COL	—	81 ±2.9	0.2 ± 0.15	20
15	Vagotomy	—	113 ± 3.5	0	80
16	Vagotomy + Me-Cl-COL	—	75 ± 2.5	1.7 ± 0.27	80
17	Crushing of intestines	—	125 ± 1.4	0	0
18	Crushing of intestines + Me-Cl-COL	—	89 ± 1.5	0.2 ± 0.15	20
19	Hemorrhage	—	117 ± 4.2	0	20
20	Hemorrhage + Me-Cl-COL	—	83 ±3.5	0	50
21	Adrenaline	600 µg	127 ± 4.9	1.0 ± 0	0
22	Adrenaline + Me-Cl-COL	400 µg	82 ± 3.5	2.1 ± 0.32	0
23	Noradrenaline	600 µg	131 ± 3.8	0.4 ± 0.40	0
24	Noradrenaline + Me-Cl-COL	400 µg	80 ± 2.5	2.9 ± 0.10	0
25	Vasopressin	30 I.U.	128 ± 2.0	0	0
26	Vasopressin + Me-Cl-COL	20 I.U.	81 ± 3.0	1.5 ± 0.40	0
27	Pentamethylenetetrazol	18 mg	134 ± 3.2	0	0
28	Pentamethylenetetrazol + Me-Cl-COL	12 mg	94 ± 2.5	0.6 ± 0.40	0
29	Diisopropylfluorophosphate	1.8 mg	105 ± 2.9	0.4 ± 0.40	20
30	Diisopropylfluorophosphate + Me-Cl-COL	1.2 mg	73 ± 2.6	1.9 ± 0.32	30
31	Na-fluoroacetate	0.15 mg	121 ± 2.5	1.2 ± 0.20	0
32	Na-fluoroacetate + Me-Cl-COL	0.10 mg	78 ± 2.8	2.0 ± 0.10	0
33	Na-iodoacetate	7.5 mg	131 ± 2.5	0	0
34	Na-iodoacetate + Me-Cl-COL	5 mg	84 ± 3.6	0	0
35	Dinitrophenol	18 mg	125 ± 2.6	0	0
36	Dinitrophenol + Me-Cl-COL	12 mg	91 ± 3.0	0	20
37	Picrotoxin	0.6 mg	136 ± 1.9	0	0
38	Picrotoxin + Me-Cl-COL	0.4 mg	93 ± 3.0	0.8 ± 0.32	0
39	Na-arsenite	1.8 mg	136 ± 2.9	0.2 ± 0.20	0
40	Na-arsenite + Me-Cl-COL	1.2 mg	92 ± 2.4	0.6 ± 0.35	0
41	Na-arsenate	7.5 mg	129 ± 4.4	0	0
42	Na-arsenate + Me-Cl-COL	5 mg	92 ± 3.0	0.4 ± 0.27	0
43	Digitoxin	2.25 mg	110 ± 3.5	0	0
44	Digitoxin + Me-Cl-COL	1.5 mg	70 ± 1.9	0	0
45	Diphterotoxin	1.5 ml	143 ± 3.0	0	0
46	Diphtherotoxin + Me-Cl-COL	1.0 ml	94 ± 1.9	0.2 ±0.15	0
47	*Pseudomonas aeruginosa* infection	2.4 ml	125 ± 4.2	0.2 ± 0.27	40
48	*Pseudomonas aeruginosa* infection + Me-Cl-COL	1.6 ml	92 ± 3.7	1.0 ± 0.36	20

* The daily dose of Me-Cl-COL (where indicated) was 500µg.

heat, cold, muscular exercise, restraint, motor denervation, spinal-cord transection, vagotomy or crushing of the intestines.

For further safety, two additional types of controls (10 rats for each) were used for each stressor agent, namely: "weight controls" (younger rats, whose mean body-weight, like that of the Me-Cl-COL pretreated animals, was 80 g, at the time of exposure to stress), and "age controls" (animals of the same age as those of the principal experiment, but whose body-weight was diminished to 80 g by partial starvation). These control groups received the same treatment as did the Me-Cl-COL-pretreated groups.However, the findings in these additional weight and age controls were essentially the same as in the not Me-Cl-COL-treated animals of the principal experiment and, hence, they are not listed in Table I.

During the experiment, the animals were fed exclusively on "Purina Fox Chow" and received tap water to drink.

On the 10th day, all survivors were killed with chloroform; their hearts were fixed in formalin and stained with our fuchsin-technique:

1) fix, for 48 hours, in 10 % formaldehyde solution neutralized by calcium carbonate; 2) dehydrate in dioxane; 3) embed in paraffin; 4) deparaffinize in xylol; 5) two changes of alcohol 95 %; 6) a few seconds in alcohol 70 % and in alcohol 50 %; 7) wash in running water, 5 minutes; 8) rinse in distilled water; 9) stain 15 minutes in *solution A* [50 ml of cresyl truly violet (stock solution = 1 g in 500 ml water and filtration after 1 hour) + 150 ml distilled water + 0.8 ml oxalic acid 1 % solution. (Must be prepared just before use.)]; 10) rinse in distilled water; 11) stain 8 minutes in *solution B* [20 ml of phosphomolybdic acid 10 % + 20 ml of phosphotungstic acid 10 % + 10 ml of acid fuchsin 2 % + 0.8 ml aniline hydrochloride 1 % + 2 ml glacial acetic acid + + 150 ml distilled water]; 12) rinse in glacial acetic acid 0.5 %; 13) differentiate in glacial acetic acid 0.5 %, 12 minutes; 14) rinse in distilled water; 15) dehydrate in alcohol 70 % and 2 changes absolute alcohol; 16) clear in xylol and mount in balsam ([30]).

The diagnosis of *cardiac necroses* was based upon the histologic findings. This is important, because, under these particular circumstances (Me-Cl-COL conditioning and subsequent exposure to stressors), even extensive necroses are often difficult to see by mere inspection with the dissecting loupe. The reason for this occasional indistinct macroscopic appearance of histologically advanced and extensive necroses is not clear. Perhaps the decomposition products that usually endow dead muscle fibers with a characteristic yellow color do not form rapidly, or perhaps they are too quickly removed, under certain conditions of experimentation. In any event, the possibility of a marked discrepancy between histologically detectable and macroscopically obvious necroses must always be kept in mind.

The lesions were graded on the basis of the microscopical examination in an arbitrary scale of 0-3, and the means of these findings (with standard errors) are listed in Table I, together with the final body weights and the percentual mortality rate.

Results

It is evident from the findings given in Table I that Me-Cl-COL alone failed to produce any cardiac necroses, however, it significantly increased the cardiotoxic effect of numerous, quite unrelated agents, particularly that of cold

(Group 4) and hot baths (Group 6), muscular exercise (Group 8), restraint (Group 10), motor denervation (Group 12), vagotomy (Group 16), adrenaline (Group 21), noradrenaline (Group 24), vasopressin (Group 26), diisopropylfluorophosphate (Group 30), Na-fluoroacetate (Group 32), picrotoxin (Group 38), and *pseudomonas aeruginosa* (Group 48). In many of the other groups, Me-C1-COL likewise increased the incidence of cardiac necroses (Groups 18, 28, 40, 42, 46), but the difference between the corticoid-treated and the corresponding control groups was less striking and not statistically significant. In the remaining groups Me-C1-COL produced no necrotic foci, but merely an increase in individual fiber necroses and fuchsinophilia; such minor effects have been disregarded in our grading.

It is possible that some of the agents failed to produce myocardial necrosis because they did not produce sufficiently severe stress at the dose levels tested. On the other hand, excessive systemic damage does not favor the production of this necrosis. In severe shock (produced in this experiment by spinal-cord transection) the pulse is weakened and the peripheral resistance is lowered to such a degree that necrosis are not elicited even when the animal is pretreated with Me-C1-COL. Despite the failure of the coronary blood supply that occurs in "artificial hibernation" induced by cold, or during cardiac arrest with K, for example, the myocardium does not show a marked tendency for necrosis.

In any event, we may conclude from the results summarized in Table I that, after Me-C1-COL conditioning, cardiac necroses can be induced by a great variety of essentially different agents; hence, the lesions are largely a nonspecific effect of stress.

The biochemical mechanism through which systemic stress thus predisposes the cardiac muscle to the production of necroses is not yet known. It is of considerable interest, however, that NaCl—which usually exerts a definite, though mild, desensitizing effect upon the production of an ESCN—augments the sensitivity of the heart to the production of necroses by stressor agents, such as forced restraint ([32]). We are now engaged in a study of this singular phenomenon, but preliminary data suggest that, in the corticoid conditioned rat, stress can so alter metabolism that even a normally inert, or actually desensitizing, Na-salt becomes an effective sensitizer.

Conclusions

The general conclusion that can be drawn from our investigations is that the myocardial necroses which occur without any morphological evidence of a vascular obstruction are largely, but not totally, nonspecific, in that they can be produced by many, but not by all, stressors. The concept of relative nonspecificity receives further support from the observation that certain factors sensitize (e.g., corticoids, Na-salts), while others desensitize (e.g., $MgCl_2$, KCl), the cardiac muscle for the production of necroses by a great variety of agents. In view of the facts just mentioned we assumed, as a working hypothesis, that nonspecific stress occupies a key position in the pathogenesis of the necrotizing cardiopathies, and that the latter could consequently be regarded as "diseases of adaptation" caused by some derailment of the general adaptation syndrome.

Our observations on the ESCN suggest that, during stress, an excessive production of corticoids or the undue activation of such steroids by certain electrolytes shifts may produce irreversible metabolic derangements in the cardiac muscle fibers, and thereby induce necrosis. The "fuchsinophilic dege-

neration" appears to be one of the earliest morphologically detectable manifestations of such biochemical disturbances in the myocardium. Inflammation is a secondary consequence of this same derangement, and, hence an integral part of the lesion. Since any sizeable focus of myocardial necrosis is secondarily invaded by inflammatory cells, it would only be misleading to distinguish sharply between cardiac necrosis and necrotizing myocarditis. Of course, inflammation may occur in the heart, as in any other organ, without being preceded by necrosis, but no matter how a necrotic focus is produced in the myocardium (e.g., by coronary occlusion, or thermocoagulation), even in the absence of any exogenous chemical or microbe, the dead tissue is destroyed and removed by inflammatory cells. We emphasize these points because they suggest that whatever may be learned about the pathogenesis and prevention of cardiac necroses will also be applicable to at least some types of myocarditis, especially to the so called "nonspecific chronic myocarditis" that eventually develops in everybody who lives long enough.

As it stands today, our hypothesis has two severe flaws: first, we have seen that several stressors (e.g., spinal-cord transection, bleeding, dinitrophenol) do not produce a necrotizing cardiopathy, even when the rats are optimally conditioned by corticoids and/or electrolytes; second, the postulated, common, metabolic derangement in the cardiac-muscle fiber, which we believe is induced by stress under certain conditions, has not yet been identified.

Our concept is not offered as a final solution, but, despite its admitted weaknesses, it seems to be the working hypothesis most compatible with all the known facts. There is overwhelming evidence in support of the view that certain conditioning factors (corticoids, Na-salts) can predispose to the production of necrotizing cardiopathies by wholly unrelated stressors. As some agents capable of producing severe stress nevertheless fail to elicit pronounced myocardial necroses, this may be a promising lead for further investigations. For example, this fact suggest experimentation to verify whether there exist adaptive mechanisms that can protect the heart against necrosis when stress of a certain intensity is produced in a certain way. The same attitude must be taken with regard to the second point, namely, our present ignorance of the fundamental biochemical derangement that causes myocardial necroses. It would be gratuitous, at present, to elaborate a detailed biochemical theory of the necrotizing cardiopathies. It is obvious, from the data summarized briefly in this paper that electrolyte shifts are important and it may be that the corticoids condition the cell by altering its affinity for certain ions—perhaps through changes in cell permeability. Our histologic observations suggest, furthermore, that some strongly fuchsinophilic material tends to accumulate in the myocardial fiber, just before necroses occurs. Whether the accumulation of the substance is the cause or the consequence of damage we do not know, but these are avenues for future research.

The question arises whether this type of biochemical conditioning, which we recently established, could play a part in the pathogenesis of the cardiac infarcts that occur in man. It has long been known that in many cases of clinical cardiac infarcts (about 30-40 %) even careful examination of the coronary vessels failed to reveal any evidence of an acute obstruction. But some atheromatosis does commonly occur, and this may produce a partial occlusion or, at least, a rigidity of the vascular wall. Such changes could interfere with the vasodilatation necessary to supply increased amounts of blood to the heart,

when its requirements for oxygen and nutrients suddenly rise (e.g., during neuromuscular stress). It is conceivable, therefore, that, at times of stress, this increase in demand could cause myocardial infarction, despite the absence of an acute coronary occclusion. Thus, the biochemical derangement produced by stress, corticoids and electrolytes, that is all-important in the production of the ESCN, may also play a role in those cardiac infarcts of man that cannot be wholly explained by coronary occlusion. This view is further substantiated by the observation that systemic stress can precipitate an ESCN in animals suitably conditioned by a corticoid, just as it can cause cardiac infarcts in predisposed human beings. If this interpretation is correct, the prophylactic measures effective in the treatment of the ESCN (e.g., KCl, $MgCl_2$) may also be beneficial in the prevention of certain infarcts.

Summary

After a brief review of our previous investigations concerning cardiopathies, more recent experiments are described that demonstrate the ability of diverse stressor agents to elicit manifestations of cardiac necrosis, in rats specifically "conditioned" for this type of response by treatment with 2α-methyl-9α-chlorocortisol (Me-Cl-COL).

Results obtained from these experiments showed that:

1) Me-Cl-COL alone failed to produce any cardiac necrosis.

2) Pretreatment with Me-Cl-COL significantly increased the cardiotoxic effect of numerous, quite unrelated agents.

3) As myocardial necroses can be elicited by such diverse agents, it may be concluded that these types of cardiac damages are a result of the nonspecific stress.

Acknowledgements

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References

(1) Bajusz, E.: *Ztschr. ges. exper. Med.*, 1958, *130*, 13.
(2) Bajusz, E.: Unpublished. Quoted by Selye, H.: *The Chemical Prevention of Cardiac Necroses*, 1958. The Ronald Press Company, New York.
(3) Büchner, F., Lucadou, W.: *Beitr. path. Anat.*, 1934, *93*, 169.
(4) DuRuisseau, J. P., Mori, K.: Symposium on "Infarctoid Cardiopathy". Montreal Phys. Soc. Meet., Montreal 7, 1958.
(5) Erb, W.: *Arch. exper. Path. u. Pharmakol*, 1905, *53*, 173.
(6) Fischer, B.: *Deutsch, Gesellsch. inn. Med.*, 1905, *22*, 235.
(7) Kellner, A., Robertson, T.: *J. exp. Med.*, 1954, *99*, 357.
(8) Kellner, A., Robertson, T.: *J. exp. Med.*, 1954, *99*, 495.
(9) Lewitzky, A.: Inaug. Diss. Petersburg, 1904. *Zeutralblat Path.*, 1905, *16*, 523.
(10) Mishra, R. K.: *Indian J. M. Res.*, 1958, *46*, 597.
(11) de Salcedo, I., Selye, H.: *Gaz. méd. port.*, 1957, *10*, 647.
(12) de Salcedo, I., *Rev. clin. espan.* 1958, *68*, 33.
(13) Schrader, G. A., Frickett, C. O., Salmon, W. D.: *J. Nutrition*, 1937, *14*, 85.
(14) Selye, H.: *Krankheitsforschung*, 1929, 7, 290.
(15) Selye, H.: *Med. Klinik*, 1929, *25*, 167.
(16) Selye, H.: *Canad. med. Ass. J.*, 1940, *42*, 113.

(17) SELYE, H.: *Stress. The Physiology and Pathology of Exposure to Stress.* Acta Inc., Montreal, 1950.
(18) SELYE, H.: *The Story of the Adaptation Syndrome.* Acta. Inc., Montreal, 1952.
(19) SELYE, H.: *The Stress of Life.* McGraw-Hill Book Co., Inc., New York, 1956.
(20) SELYE, H.: Laurentian Hormone Conf. Mont-Tremblant, September 1-6, 1957.
(21) SELYE, H.: *Amer. Heart J.*, 1958, *55*, 1.
(22) SELYE, H.: *Folia clin. internac.*, 1958, *8*, 97.
(23) SELYE, H.: *Acta endocr.*, (Copenhagen) 1958, *28*, 273.
(24) SELYE, H.: *Endocrinol. e sc. costit.*, 1958, *24*, 345.
(25) SELYE, H.: *Brit. Med. J.*, 1958, March 15, 599.
(26) SELYE, H.: *Circulation Res.*, 1958, *6*, 168.
(27) SELYE, H.: *Internat. Arch. Allergy*, 1958, *12*, 145.
(28) SELYE, H.: *Angiology*, 1958 (in press).
(29) SELYE, H.: *Proc. Soc. exp. Biol.*, N. Y., 1958, *98*, 61.
(30) SELYE, H.: *The Chemical Prevention of Cardiac Necroses*, 1958. The Ronald Press Company, New York.
(31) SELYE, H., BAJUSZ, E.: *Cardiologia*, 1958 (in press).
(32) SELYE, H., BAJUSZ, E.: Unpublished. Quoted by Selye, H.: *The Chemical Prevention of Cardiac Necroses*, 1958. The Ronald Press Company, New York.
(33) SELYE, H., MISHRA, R. K.: *Amer. Heart J.*, 1958, *55*, 163.
(34) SELYE, H., PENTZ, I.: *Canad. med. Ass. J.*, 1943, *49*, 264.
(35) SELYE, H., RENAUD, S.: *Presse méd.*, 1956, *66*, 99.
(36) SELYE, H., RENAUD, S.: *Proc. Soc. exp. Biol.*, N. Y., 1957, *96*, 512.
(37) SELYE, H., RENAUD, S.: *Exper. Med. & Surg.*, 1957, *15*, 335.
(38) SELYE, H., RENAUD, S.: *Amer. J. Cardiol.*, 1958, *1*, 208.
(39) SELYE, H., RENAUD, S., NÁDASDI, M.: *J. Pharmacol. & exp. Therap.*, 1958, *122*, 504.
(40) SELYE, H., RENAUD, S., NÁDASDI, M.: *Endocrinology*, 1958, *62*, 541.
(41) SELYE, H., DE SALCEDO, I.: A.M.A. *Arch int. Med.*, 1958 (in press).
(42) ZSOTER, T., PINTER, I., NÁDASDI, M.: Symposium on "Infarctoid Cardiopathy". Montreal Phys. Soc. Meet., Montreal, March 7, 1958.

ON THE RELATIONSHIP BETWEEN END SYSTOLIC VOLUME AND VENTRICULAR MECHANICAL IMPULSE—A NEW LAW OF THE HEART

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IF SUFFICIENT allowance is made for differences, the viscoelastic theory of the muscular contraction is capable of yielding a fair interpretation of the cardiac activity. This theory must be revised ([1, 5, 9, 10]) so as to comprise ideas starting from Fenn's ([4]) and Stella's ([8]) papers, but it is permissible to leave aside any correction when not too precise aspects of the physiological problem are considered. On this basis, according to "Starling's laws" ([7]), the *end diastolic volume* has a decisive importance as far as the energy of contraction and the functional reserve of the heart is concerned. A less well-known point of the cardiac activity is the determinism of the *end systolic volume*. Some aspects of this problem have been analyzed by Holt ([6]) who has related the end systolic volume to end systolic pressure. The present paper deals with the relationship between end systolic volume and ventricular mechanical impulse; it is based on a comparative study of the pressure-time areas of two successive ventricular contractions: one *iso*-(or *auxo*-) tonic-isometric and the other totally isometric at the end volume of the first one.

EXPERIMENTAL METHOD

The technique is based on a similar one published previously ([2, 8]) and is self-explained in figure 1. The isolated toad ventricle is ligated in the atrial-ventricular groove to the neck of a glass funnel *A*, so that a common ventricular-funnel cavity is established. The funnel is wedged into another glass reservoir *B*, completely filled with Ringer solution. Stopcock *C* allows complete closure of this ambient, or its communication, by means of a three-way stopcock *D*, either with the burette *E* or with the finely graduated syringe *F*. The perfusion liquid is introduced in *A*, up to the thick part of the funnel (3 cm ID.), and is made of blood taken from the aorta of the same animal, heparinized, and 50 % diluted with Ringer solution. Electrodes *g* excite the ventricle regularly, with instantaneous electrical shocks. This dispositive permits complete experimental control of the perfusion fluid, its reduction to a minimum, and its continuous oxygenation. The pressure curve obtained with manometer *M* is equal, but inverted, with regard to the intraventricular pressure curve. Calibration of manometer *M* must be done "in negative"; the *O* level coincides with the funnel meniscus. The

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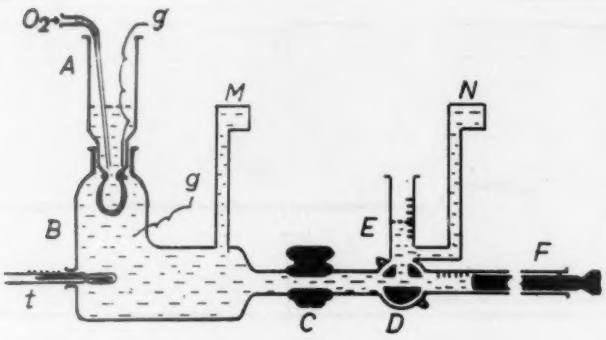

FIG. 1. — *Diagram of the experimental set-up. See text.*

sensitive manometer *N* records ventricular volume variations. Syringe *F* allows the calibration of this manometer, and the measurement of the end systolic volume. When stopcock *C* is closed, heart contraction is isometric; when reservoir *B* communicates with the burette it is *iso*-(or *auxo*-) tonic (this depends on the resistance in *D*); when stopcock *C* is closed in the course of a contraction, a two-phase, *iso*-(or *auxo*-) tonic-isometric contraction is obtained. Optical and electromanometers were employed. Heating or cooling reservoir *B* allows experiments to be performed at different temperatures, thereafter a good approximation has been reached between the thermometer *t*, and another one introduced in the perfusion fluid.

RESULTS AND DISCUSSION

Figures 2 and 3 reproduce the pressure and volume records of some experimental sequences. Pressure curves are inverted because of the technique employed. A first fact to consider is that contractions *4* and *5* are equal, irrespective of the fact that they follow contractions of different type. This shows that in a regular series of contractions, the mechanical characteristics of each one does not influence the following one.

Figure 4 results from the chronological superposition of three contractions, starting with excitation at instant *O*; namely: *V*) an isometric one at volume *V*; *Vv*) an initially isotonic (volume *V* to *v*) and thereafter isometric (volume *v*); *v*) a totally isometric at volume *v*. Curves *v* and *Vv*, after instant *t*, are isometric and correspond to the same volume *v*, but differ in the mechanical conditions during the interval *O-t*. It is evident that the relative position of the isometric segments, their crossing and the areas *I* and *II*, are a consequence of those different mechanical conditions. The loss of pressure which is represented by area *I* can be imputed to viscosity, but it is difficult to explain in the same way the small area II. The unknown cause which determines areas *I* and *II* will be designated as "hysteresis". Figure 5 shows that area *I* decreases for either too long or too short isotonic intervals. Figure 6 shows that temperature tends to increase area *II* at the expense of area *I*. Apart from these purely qualitative relationships, no other simple relationship seems to arise when the two isometric segments are compared.

In the same figure 4 two other areas of pressure-time are limited; these are: area *x* between curves *V* and *v* and area *y* between curves *V* and *Vv*. With reference to the isometric contraction *V*, area *x* is the loss of pressure-time due

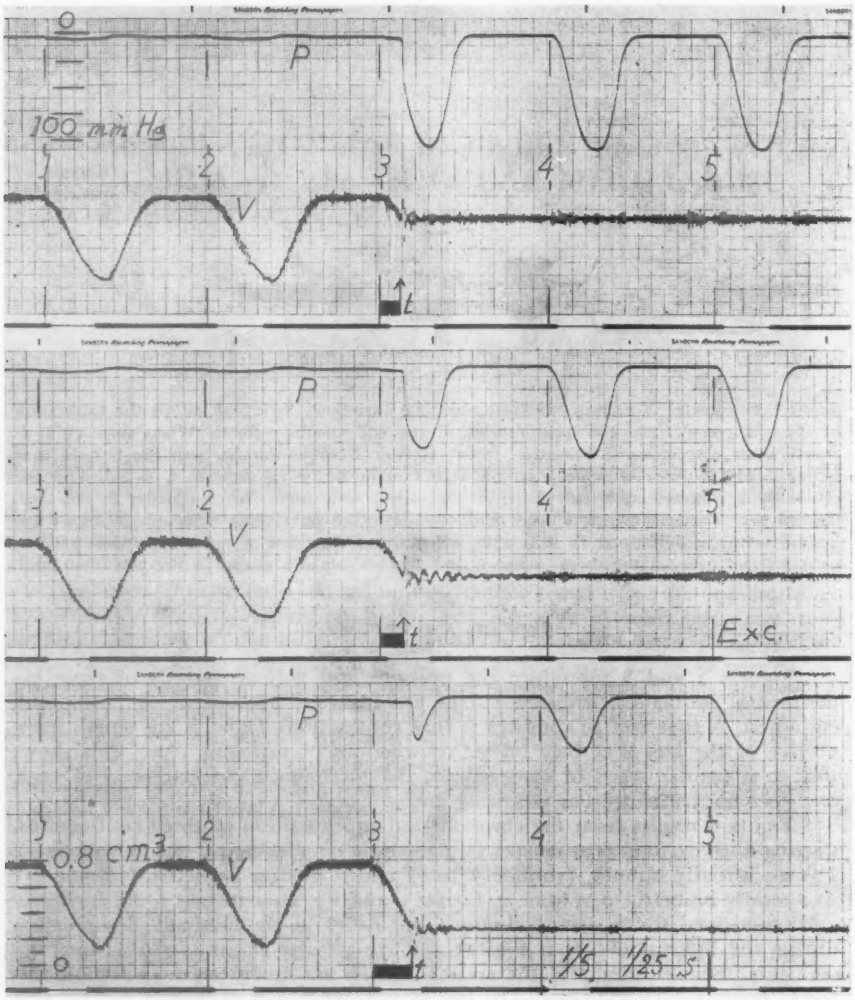

FIG. 2. — *Three typical experimental sequences.* P: *pressure curve (inverted);* V: *volume curve. Numbers indicate the instant of the excitation referred to the signal at the bottom). Contractions* 1 *and* 2 *are isotonic. Contraction* 3 *is isotonic up to instant* t *and thereafter is isometric. Contraction* 4 *and* 5 *are isometric at the end volume of contraction* 3. *Important differences between areas* 3 *and* 4 *are observed when the isotonic interval* varies (horizontal bar).

to a determinate reduction of the isometric contraction volume, and area *y* is the loss of pressure-time due to the same reduction of volume, provided it occurs during an initial, isotonic phase of the same contraction.

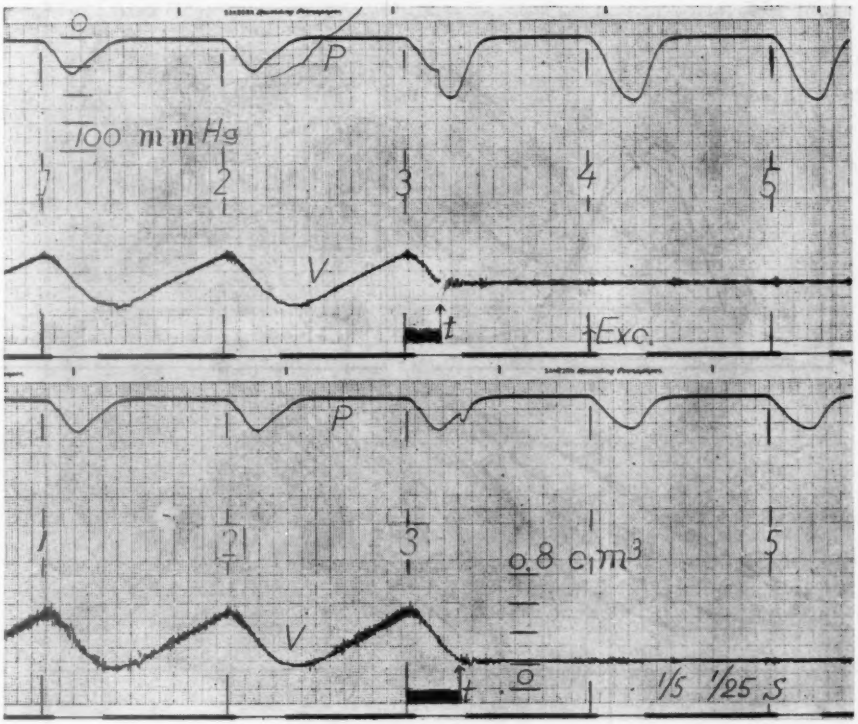

FIG. 3. — *Two sequences as in fig. 2, but contractions 1 and 2 are auxotonic and contraction 3 is auxotonic-isometric. Great variations of the auxotonic interval produce scarce differences between areas 3 and 4.*

In figure 7 areas *y* are plotted against areas *x* for each experimental sequence. All the sequences belong to two ventricles at different temperatures and with different isotonic intervals and are grouped in seven ventricle-temperature series. The areas are expressed in percents of the greatest area *x* of each series. All points are contained in a narrow band, immediately above the 45° inclined line passing through *"O"* (line of equal areas) and describe a curve of slight downward concavity. Obviously as the isotonic interval tends towards O, areas *x* and *y* tend toward O; as the isotonic interval increases, areas *x* and *y* approach the limit imposed by area *V*. Between those limits, points maintain themselves close to the line of equal areas. In a further analysis, the lineal functions corresponding to the 7 ventricle-temperature series have been calculated by the least squares procedure. The seven straight lines have a next to 45° inclination, and a small ordinate at the origin. They, thus, reiterate for each group of points, the same tendency of the whole. Functions seem not to

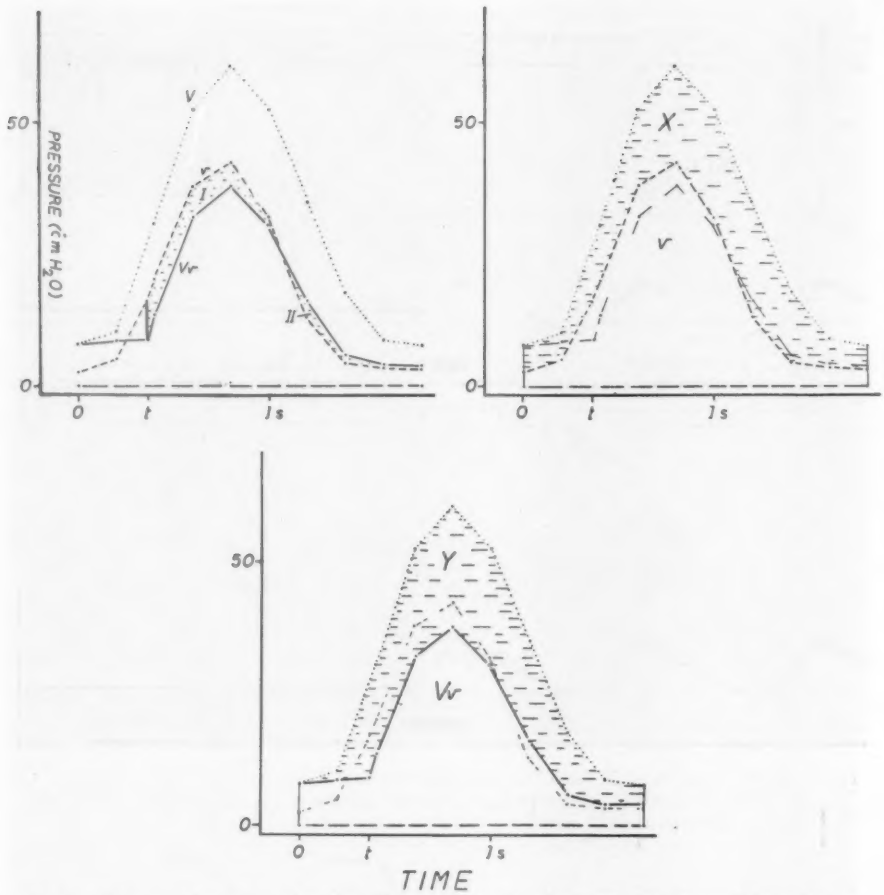

FIG. 4. — *Superposition of contractions* V *(isometric at volume* V*),* Vv *(isotonic-isometric at volume* V *to* v*),* v *(isometric at volume* v*). See areas* V, Vv *and* v*; areas* ($x = V - v$) *and* ($y = V - Vv$)*; and areas* I *and* II. *See text.*

differ on account of temperature and, consequently, this factor does not influence the relationship between areas x and y. It can be deduced from these results a clear tendency towards equality of areas x and y, and, at the same time, a systematic cause maintaining a certain difference in favor of areas y. A simple transformation shows that the relationship between areas x and y depends exclusively on that between areas v and Vv (fig. 4).

In fact, from $V - v \leqq V - Vv$

it results $v \geqq Vv$ (*)

(*) It is for purely circumstantial reasons that in figure 7, it is areas x and y, while in figure 8, it is areas v and Vv which were compared.

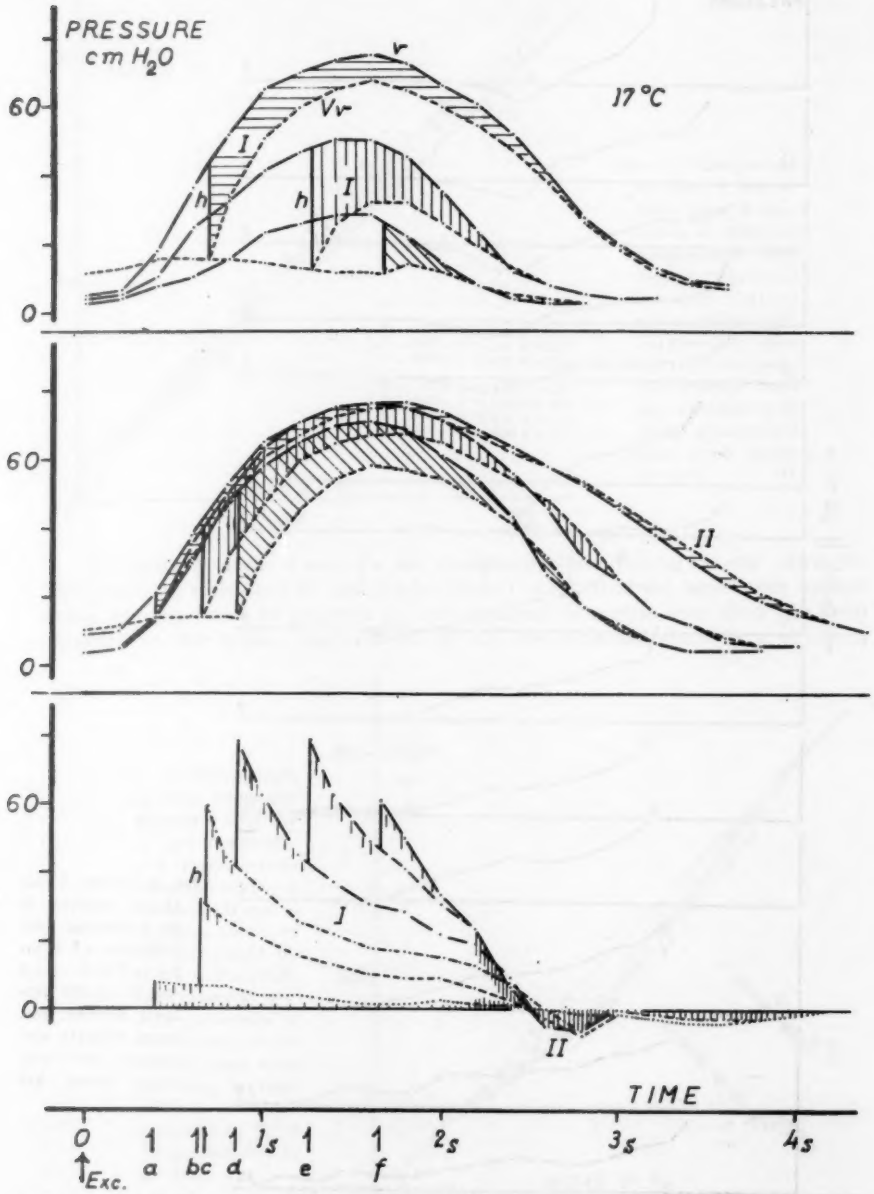

FIG. 5. — *Areas* I *and* II *in 6 experiments with different isotonic intervals. Bottom: algebraic sum of the areas. See text.*

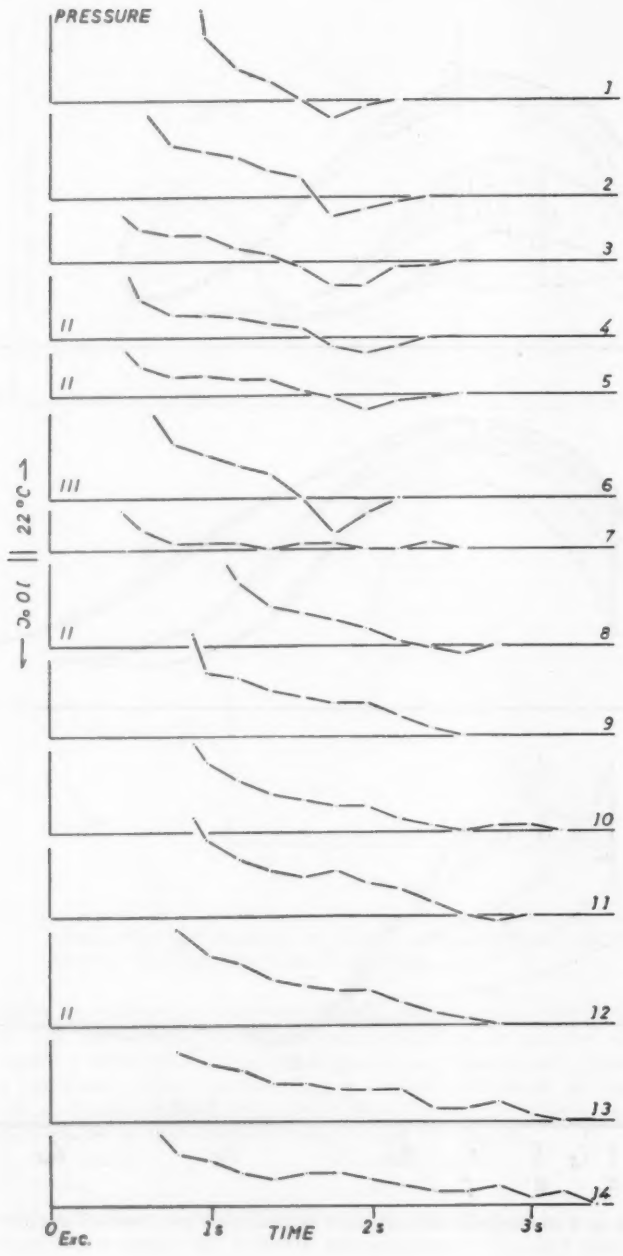

FIG. 6. — *Areas* I *and* II *are referred to an horizontal axis. Influence of temperature and length of the isotonic interval. Romans indicate average of 2 or 3 very close curves. See text.*

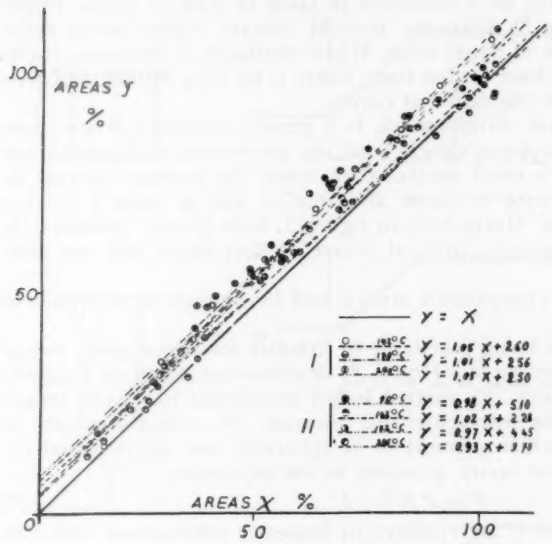

FIG. 7. — *Comparison of areas* x *and* y. *Data from 2 ventricles at different temperatures, with different isotonic intervals. Curves and equations correspond to each ventricle - temperature group. Areas are expressed in % of the greatest area* x *of each group. See text.*

In figure 8, areas *v* and *Vv* are compared. Data belong to one ventricle. Temperature, resistance *D* and *iso*-(or *auxo*-) tonic interval have been varied. Areas are expressed in percents of the maximal isometric area that has been obtained at the lowest temperature (9° C). As expected, there is a tendency

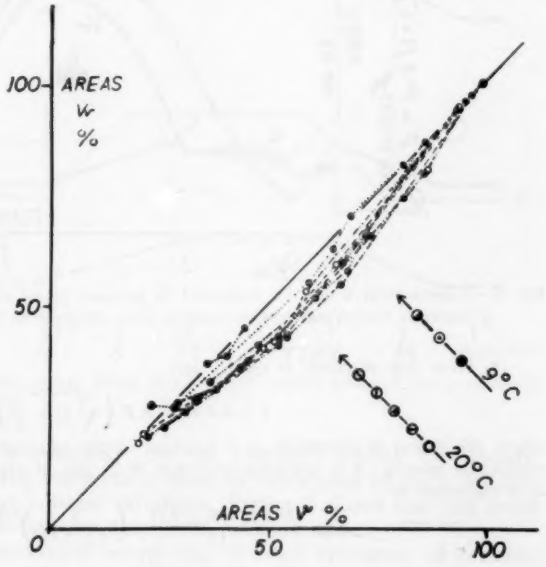

FIG. 8. — *Comparison of areas* Vv *and* v. *Data of a ventricle at two temperatures. Each curve corresponds to a resistance D and to different values of the auxotonic interval. Arrows indicate expected relative proximity of the curves to the line of equal areas according to resistance* D. *Areas are expressed in % of the greatest area* x. *See text.*

towards the equality of areas an a difference in favor of area v. Points corresponding to each resistance D determine upward concave curves whose extremities tend to join the line of equal areas. When resistance D increases, curves are shorter and are placed closer to that line. There is no clear influence of temperature on the position of the different curves.

The fact that the curves corresponding to a greater resistance D are closer to the line of equal areas, appears clearly from the comparison of figures 2 and 3. In fact, in fig. 2, with a small resistance D, when the isotonic interval interval increases, the difference in shape and relative size of areas 3 ($= Vv$) and 4 ($= v$) also increases. Meanwhile, in figure 3, with greater resistance D, large variations of the auxotonic interval, scarcely affect shape and size similarities of areas 3 and 4.

Two corrections tend to approximate areas v and Vv, though in very reduced terms, namely:

a) It can be claimed as being preferable to compare force-time areas instead of pressure-time areas. According to a Holt's (6) development, based on Laplace's equation, the tangential force (F) exerted by all ventricular fibres can be calculated as a function of the intraventricular pressure (P), the radius (R) of the ventricular cavity, which is assumed to be spherical, and the maximal circumference (C) of the same cavity, acording to the expression

$$F = P\,R\,C\,/\,2 \qquad (*)$$

It is understood that R and C are constant in isometric contractions and, consequently, force and pressure curves are superposable (provided a convenient

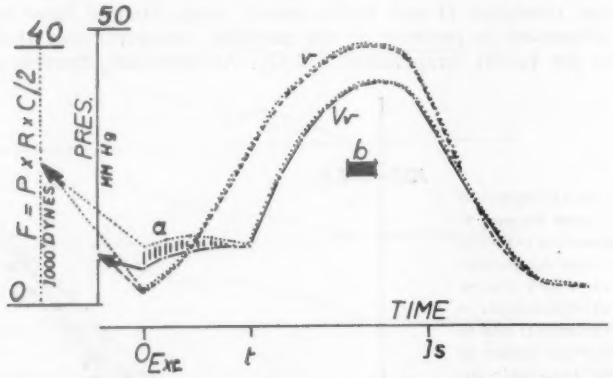

FIG. 9. — *Contractions* v *and* Vv *expressed in pressure (solid and broken lines) and in force of a maximal ventricular section (dotted line). Amount of corrections* a *and* b. *See text.*

(*) From that equation, it results that

$$F = k\,P\,R^2 = K\,P\left(\sqrt[3]{Q + \frac{q}{2}}\right)^2$$

where the radius is calculated as a function of the internal ventricular volume Q and the ventricular mass q (F is expressed in dynes; P in mm of Hg; Q in cm^3; q *in* gm). Constant K is expressed by

$$K = \frac{13.6 \times 980 \times 3.14}{10}\left(\frac{3}{4 \times 3.14}\right)^{2/3} = 1610.5$$

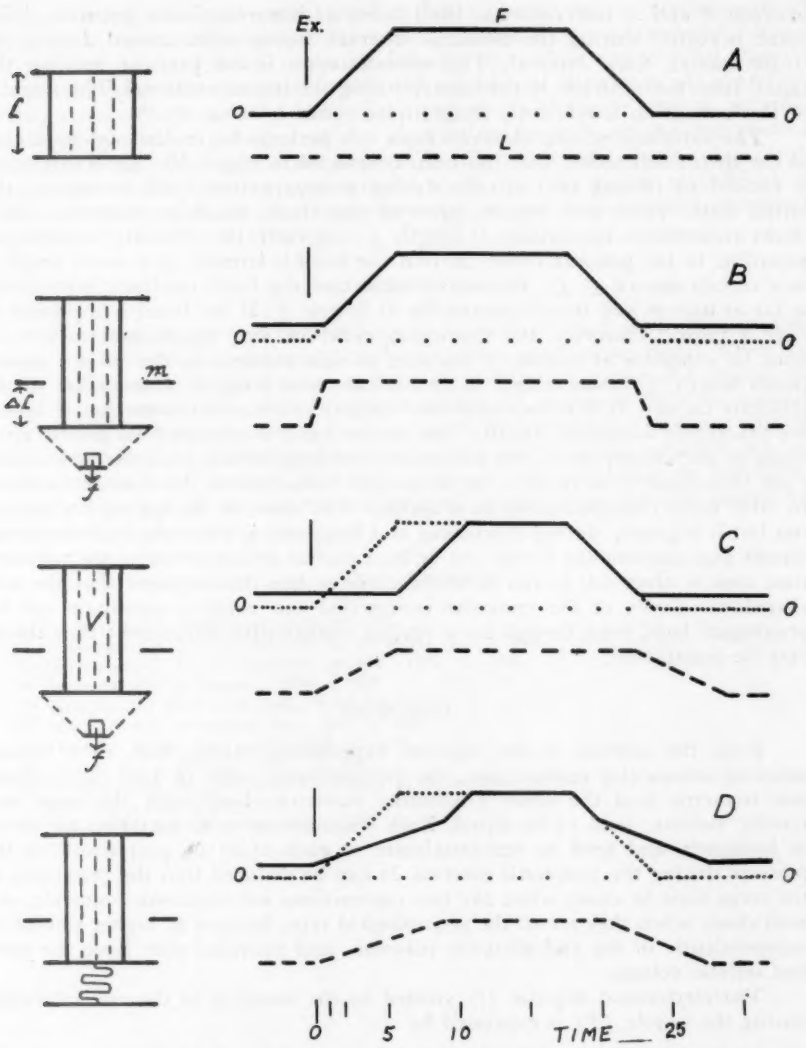

FIG. 10. — *Theoretical model. Force curves (solid line); length curves (broken line); isometric reference curve (dotted line).*

ordinate scale relationship is adopted). On the contrary, during the isotonic interval of contraction *Vv*, the force curve must be calculated in each instante on account of the ventricular volume variations. Figure 9 shows that the small area *a* is the only difference between force and pressure areas.

b) In fact, in the experimental set-up (fig. 1), the difference of pressure

between *B* and *A* reservoirs has been taken as intraventricular pressure. This value is correct during the isometric interval, but is subestimated during the *iso*-(or *auxo*-) tonic interval. This subestimation is the pressure moving the liquid from the ventricle to the funnel during the isotonic interval. The amount of this correction is relatively insignificant (area *b* in fig. 9) (*).

The rationale of the observed facts can perhaps be understood by means of the theoretical model that has been developed in figure 10. An elastic *band* is excited in instant *O*, contracts during a certain time, and returns to the initial state. Force and length curves of the elastic band are recorded. 10-*A* shows an isometric contraction at length ℓ. Logically, the elasticity factor varies according to the pressure curve. In 10-*B* the band is stressed by a small weight *f* in a certain stretch $\triangle \ell$. Because of excitation the band contracts isotonically as far as tops *m* and then isometrically at length ℓ. If the band is supposed to have a perfect elasticity, the shortening must be very rapid; for instante, it must be complete at instant, *1*, because, in this moment to the force *f* corresponds length ℓ (more length in this instant would signify a reduction of the elasticity factor). It is to be noted that force-time areas of contraction *A* and *B* are practically identical. In 10-*C* the elastic band is supposed to possess great viscosity and consequently, the shortening and lengthening intervals under force *f* are prolonged. The result is an important reduction of the contraction area. In 10-*D* force *f* is substituted by a spring. The stress of the spring is *f* during rest but it is greater during shortening and lengthening intervals; it prolongs the former and shortens the latter. An at least partial recuperation of the pressure-time area is observed. It can be deduced from this development that the sole viscoelastic theory of the muscular contraction can roughly anticipate the experimental facts, even though for a perfect quantitative adjustment that theory may be insufficient.

Comments

From the analysis of the reported experiments results that, in a regular series of ventricular contractions, the pressure-time areas of two contractions, one isometric and the other auxotonic- isometric, both with the same end systolic volume, tend to be equal. Both contractions tend to differ by virtue of hysteresis, and tend to reapproximate to each other in proportion to the pressure during the auxotonic interval. It can be deduced that the proximity of the areas must be closer when *the two* contractions are auxotonic-isometric, and even closer when they are of the physiological type, because of higher similarity, independently of the end diastolic volumen, and provided they have the same end systolic volume.

The mechanical impulse (*J*) yielded by the ventricle to the arterial system during the systole (*T*) is expressed by

$$J = S \int_0^T P \, dT$$

(*) The mean pressure *P* (mm Hg) which moves the intraventricular liquid *M* (cm^3) through the section *S* (cm^2) funnel hole, during the isotonic interval *T* (s) has been calculated by the formula:

$$P = K M / T^2 S^2$$
$$(K = 10 / 13.6 \times 980 = 1 / 1333)$$

where S represents the arterial cross-section supposed to be constant, and the rest, the intraventricular pressure-time area. As this area is a biunivocal function of the end systolic volume, it can be deduced that, *when any physical, chemical or physiological variation is excluded, the mechanical impulse of the ventricular contraction depends on the end systolic volume*, in a similar way as, according to Starling's law, the energy of the contraction depends on the end diastolic volume. Both relationships are implicit in the viscoelastic theory of muscular contraction, and have an approximate scope. It rests to be established the function which rules the dependence between pressure-time area and end systolic volume.

Summary

In a regular series of contractions of an isolated toad ventricle, autotonic-isometric and totally isometric contraction areas tend to be equal, when they have the same end systolic volume. Both areas differ by virtue of hysteresis and tend to approach each other in proportion to the auxotonic interval pressure.

It can be deduced that two contractions of physiological pattern, with the same end systolic volume (irrespectively of the end diastolic volume), are very similar from their pressure-time area point of view and that the mechanical impulse of a ventricular contraction depends primarily on the end systolic ventricular volume.

Bibliography

(1) Aubert, X.: *J. de physiol.*, 1956, *48*, 105.
(2) Duomarco, J. L., Beovide, H. S., Esponda, A. C.: *Rev. argent. cardiol.*, 1956, *23*, 117.
(3) Duomarco, J. L.: *Rev. argent. cardiol.*, 1947, *13*, 320.
(4) Fenn, W. O.: *J. Physiol.*, 1924, *6*, 373.
(5) Hill, A. V.: *Brit. med. Bull.*, 1956, *12*, 174.
(6) Holt, J. P.: *Circulation Res.*, 1957, *5*, 281.
(7) Starling, E. H.: *Pres. med.*, 1922, *60*, 641.
(8) Stella, G.: *J. Physiol.*, 1931, *72*, 247.
(9) Szent-Györgi, A.: *Chemical Physiology of contraction in body and heart muscles.* Academic Press, New York, 1953.
(10) Wilkie, D. R.: *Brit. med. Bull.*, 1956, *12*, 177.

CHRONIC ARTERIAL HYPERTENSION IN HEMINEODECORTICATE RATS

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It has been shown that hemidecortication in rats results in a transient arterial hypertension that falls temporarely by the administration of sympathicolytic drugs (1).

In an attempt to localize the cerebral region responsible of this hypertension, rats with ablation of neocortex and archicortex were studied.

Material and methods

White rats weighing 150-200 g were used. The operating procedure has been described previously (1). In the present work the neocortex was sucked away in one group of 38 rats and the archicortex in another group of 60 rats, both on the left side. Figures 1 and 2 show the operation performed. Arterial blood pressure was measured weekly by the method of Williams, Harrison and Grollman (2). Rats with blood pressure over 120 mm Hg were considered hypertensives. Every group was compared with a group of control rats that underwent a sham operation.

Blood pressure was determined during 20 weeks after the operations; in one group of hemidecorticate rats the remaining neocortex was sucked away at 20 weeks after the first operation and blood pressure measured during another period of 20 weeks.

Results

Hemineodecortication: In this group the survival was 100 %. The rats lost 12 g of weight on the average during the firts 3 days, but it was regained in 10 days. Twenty four of the operated rats (63 %) developed hypertension; the average value and the fiducial limits of the mean of these rats are represented in figure 3 (full line) compared with 34 controls (broken line). It can be seen that 3, 2 and 1 week before the operation the average value was 106, 108 and 106 mm Hg for 72 rats. The rats with removal of neocortex developed hyper-

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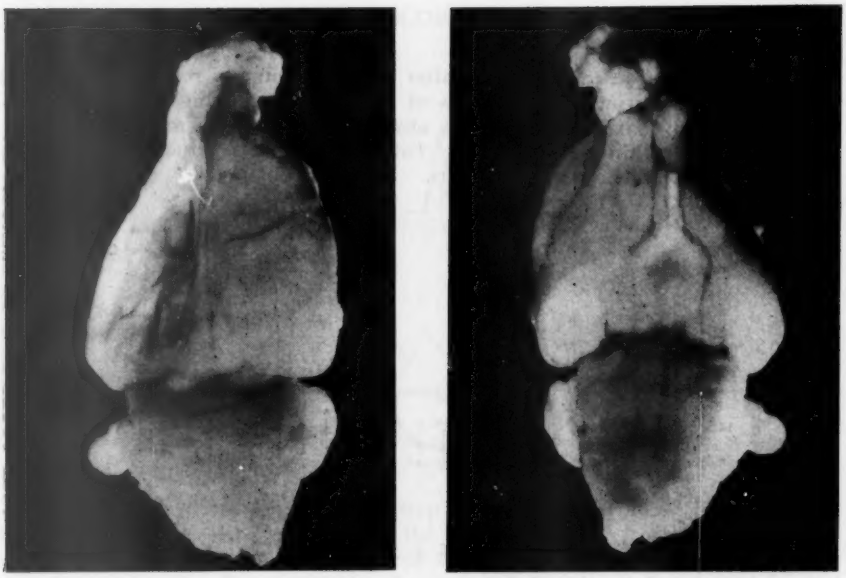

FIG. 1. — *Photographs to illustrate the hemineodecortication performed. On left: dorsal view. On right: ventral view.*

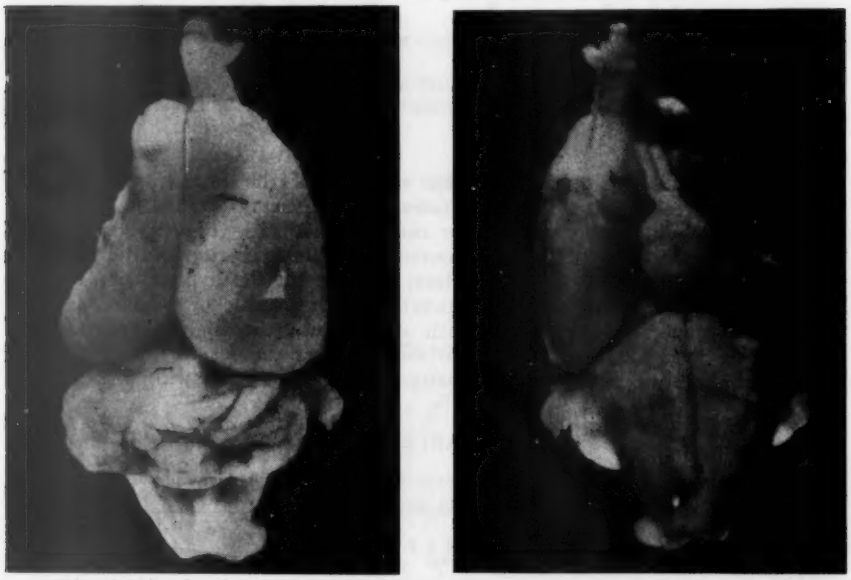

FIG. 2. — *Photographs to illustrate the hemiarchidecortication performed. On left: ventral view. On right: dorsal view.*

tension that started at the first week after the operation and rose gradually and was maintained during the 20 weeks of observation, the higher value being 141 mm Hg. No sexual difference was observed. Control rats maintained their blood pressure within normal values. Table 1 shows the values of the blood pressure obtained in both group of rats.

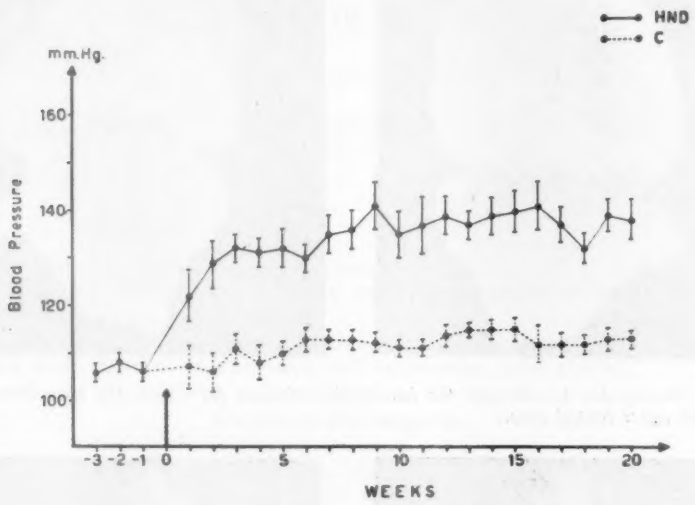

FIG. 3. — *Average blood pressure and its fiducial limits of 24 hemidecorticate rats (HND) (full line) and 34 control rats (C) (broken line). Arrow shows time of hemidecortication and craneotomy.*

In the previously operated group, removal of the remaining neocortex was made 20 weeks after the first operation. There was no change in the blood pressure either in the hypertensive or normotensive rats. Figure 4 shows the result obtained in one rat that underwent a double operation (full line) and their correspondent control (broken line). In this rat the hypertension started on the second week after the first operation with a value of 155 mm Hg and then maintained its high values with oscillations, the highest value being 175 mm Mg reached at 12, 16 and 20 weeks. After the second operation was performed there was no significant change during the 20 following weeks.

TABLE I

Changes in blood pressure of hemineodecorticate (AND) and hemiarchidecorticate (HAD) rats compared with controls (C) (mmHg)

GROUP	Nº of rats	WEEKS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
HND	24	122	129	132	131	132	130	135	136	141	135	137	139	137	139	140	141	137	132	139	
C	34	107	101	111	108	110	113	113	113	112	111	111	114	115	115	115	112	112	112		
HAD	24	100	105	105	103	96	95	99	93	101	105	104	107	111	111	104	105	108			

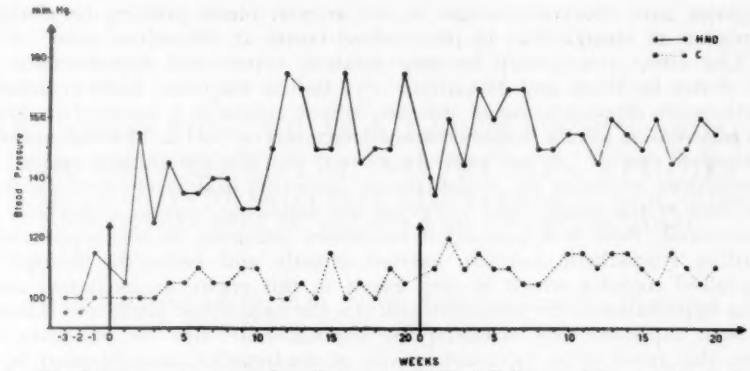

FIG. 4. — *Blood pressure of one neodecorticate rat (HND) (full line) and its control (C) (broken line). First arrow: left neodecortication. Second arrow: right neodecortication.*

Hemiarchidecortication: In this group the survival was 40 % The rats lost 23 g of weight on the average during the first 3 days and from then on, they regained it gradually in 20 days. The blood pressure changes are shown in figure 5 (full lines) where it can be observed that there was a transient hypotension between 5 and 10 weeks, the lowest value being 93 mm Hg at 8 week. Control rats are represented in broken lines. Table 1 shows the value of the blood pressure of this group of rats.

DISCUSSION

The role that the cerebral cortex plays in the regulation of the cardiovascular system is known since the works done by Schiff [3]. Since then many in-

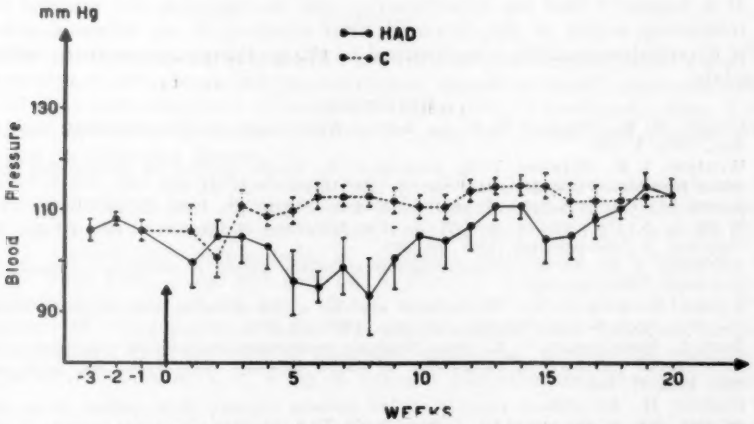

FIG. 5. — *Average blood pressure and its fiducial limits of 24 hemiarchidecorticate rats (HAD) (full line) and 34 control rats (C) (broken line). Arrow shows time of hemiarchidecortication and sham operation.*

vestigators have observed changes in the arterial blood pressure by electrical stimulation of several areas of the cerebral cortex in animals or man.

The direct relationship between cerebral cortex and hypothalamus has been shown by Ward and McCulloch (4), and on the other hand evidence of hypothalamic representation of the sympathetic system is a known fact. Unilateral stimulation of the hypothalamus determines a rise in blood pressure in anesthetized cats (5). It has been also proved that the amigdaloid complex has a modulatory action on the hypothalamus according to different authors (6, 11).

In view of the results here presented the following working hypothesis can be advanced: there is a neocortical inhibitory influence on the hypothalamus regarding sympathetic function exerted directly and indirectly through the amygdaloid complex which in turn exerts in this regard an excitatory action on the hypothalamus. In neodecorticate rats the neocortical inhibitory influence has been supressed thus releasing the hypothalamus and the amigdala, two factors that result in an increased activity of the hypothalamus, revealed in this case by an increased arterial blood pressure. The presence of the permanent excitatory action of the amygdala would account for the chronic hypertension and its absence in the hemidecorticate rats would explain the transitoriness of the hypertension previously reported.

Summary

Arterial hypertension developed in 63 per cent of hemineodecorticate rats; it started in most of them on the week following operation and lasted up to the end of the experiment (20 weeks).

The neodecortication of the remaining hemisphere did not cause any change in blood pressure: the already hypertensive rats remained hypertensive without significative change and the normotensive did not increase its blood pressure. The study during 20 weeks after the second operation increased up to 40 weeks the time of observation of one group of hipertensive rats.

Hemiarchidecortication results in a transient fall of the blood pressure between the 5 and 10 weeks following operation.

It is suggested that the hypothalamus and the amygdala are released from the restraining action of the neocortex thus resulting in an increased activity of the hypothalamus which is maintained by the excitatory action of the released amygdala.

References

(1) Covian, M. R., Houssay, H. E. J.: *Arterial hypertension in hemidecorticate rats.* Circ. Res., 1955, *3*, 459.

(2) Williams, J. R., Harrison, T. R., Grollman, A.: *Simple method for determining systolic blood pressure of unanesthetized rats.* J. clin. Invest., 1939, *18*, 373.

(3) Schiff, M.: Cit. by Schäfer, E. A. *Text-book of Physiology*, 1900, *2*, 714.

(4) Ward, A. A. (Jr.), McCulloch, W. S.: *The projection of the frontal lobe on the hypothalamus.* J. Neurophysiol., 1947, *10*, 309.

(5) Schneider, J. A.: *Further characterization of central effects of reserpine (Serpasil).* Am. J. Physiol., 1955, *181*, 64.

(6) Klüver, H., Bucy, P. C.: *Preliminary analysis of functions of the temporal lobes in monkeys.* Arch. Neurol. Psychiat., Chicago, 1939, *42*, 979.

(7) Bard, P., Mountcastle, V. B.: *Some forebrain mechanisms involved in expression of rage with special reference to suppression of angry behavior.* Res. Publ. Ass. nerv. ment. Dis., 1947, *27*, 362.

(8) Gastaut, H.: *Corrélations entre le système nerveux végétatif et le système de la vie de relation dans le rhinencéphale.* J. Physiologie, 1952, *44*, 431.

(9) Gloor, P.: *Electrophysiological studies on the connections of the amygdaloid nucleus in the cat (Part II).* ELG Clin. Neurophysiol., 1955, *7*, 243.

(10) Schreiner, L., Kling, A.: *Rhinencephalon and behavior.* Amer. J. Physiol., 1956, *184*, 486.

THE DOMESTIC CAT AS A LABORATORY ANIMAL FOR EXPERIMENTAL NUTRITION STUDIES

V. – SOME DATA ON NUTRITIONAL REQUIREMENTS, BIOCHEMICAL AND PHYSIOLOGICAL VALUES AND ORGANS WEIGHT

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ALTHOUGH the domestic cat has been largely used as an experimental animal, information on biological values is very scarce; some valuable references were collectected by Albritton ([1, 2]); the endocrinology of reproduction has been reviewed by Dawson ([13]).

Dickson and Scott ([14, 15]) studied the adequate protein level for growth and reproduction and reviewed the literature on this subject.

On the sequence of our experiments about the nutritional requirements of this species, we have had the opportunity to collect a reasonable amount of data most of them on purified diets.

Even though they are not complete, we feel that they should be offered to other investigators in order to facilitate their work.

METHODS

The handling of the animals, the growth rate and hematological values on stock and purified rations and the techniques to collect blood, feces and urine, have already been described (Carvalho da Silva ([7, 8]); Carvalho da Silva, Fried and De Angelis ([9]); Carvalho da Silva, De Angelis, Pontes and Guérios ([10]); Carvalho da Silva and Pontes ([11]).

The stock and purified rations used to collect the data reported on the present publication have the following percentage compositions; *stock ration:* crude casein, 10; compressed oats, slightly cooked, 20; cooked and mashed potatoes, 15; ground lean beef muscle, 20; sardines, eviscerated and steamed, 20; lard, 10; bone meal, 2 and cod liver oil, 3. *Purified ration:* vitamin free casein, 35; gelatin, 2; peanut oil, 10; lard, 10; salt mixture IV (Phillips and Hart) ([24]), 5; sucrose, 38. The purified ration was supplemented with vitamins administered separately. One mg each of thiamine, riboflavin, pyrydoxine, vitamin K and para-amino-benzoic acid; 4 mg of calcium pantothenate; 10 mg of niacin;

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0.5 mg of folic acid; 0.5 mg of d-biotin; 20 mg of inositol and 300 mg of choline were dissolved in 1 ml of water, emulsified in 2 ml of cod liver oil and the mixture given by mouth to each animal three times a week; once a week, 15 mg of alpha tocopherol were added.

The following chemical methods were used: blood glucose (Folin and Svedberg) ([17]); plasma proteins, by microkjehldal, with distillation over 4 % boric acid and back titration with 0.02 N SO_4H_2 (Hawk, Oser and Summerson) ([22]); blood urea: acid hydrolysis (Cavalcanti and Sonnleithner) ([12]); blood nonprotein nitrogen (Folin and Wu) ([18]); plasma calcium (Halverson and Bergeim) ([21]); plasma inorganic phosphorous (Bodansky) ([4]); plasma and red cells chorides (Eisenman) ([16]); blood total cholesterol (Grigaut) ([19]); plasma potassium and sodium* (Hald) ([20]); plasma, red cells and adrenal ascorbic acid (Roe and Kuether) ([26]); red cells DPN (Levitas, Robinson, Rosen, Huff and Perlzweig) ([23]); plasmatic iron (Teixeira Mendes and Germeck) ([27]); blood pyruvic acid (Bueding and Wortis) ([6]); blood lactic acid (Barker and Summerson) ([3]); total liver and fecal fat (Van de Kamer, Bokkel Huinink and Weyers) ([28]).

Plasma and blood volumes were determined by the Evans blue (Carvalho da Silva, De Angelis, Pontes and Guérios) ([10]); total water volume was evaluated by the antipyrine method (Brodie) ([5]); 50 mg of antipyrine per kg of body weight, were dissolver in 5 % glucose, and injected into the peritoneum; blood samples were collected from the saphena magna, before and 1 - 1,5 - 2 and 2,5 hours after the injection (Carvalho da Silva and Pontes) ([11]).

Results

a) *Protein requirements.* The influence of the casein level of purified rations (22 %, 35 % and 45 %) was studied on a group of 17 growing cats, during 21 days (Table I). The variations on the protein level were compensated by modification of the sucrose percentage, on a weight basis. It is evident from table I, that 22 % casein is inadequate for growth but 35 or 45 % casein produce identical results.

In accordance with these high protein requirements it was observed that

TABLE I

Weight gains of growing cats after 21 days on purified rations, with 22 %, 35 % and 45 % of casein

Group	Number of animals	Casein level	Initial body weight (g)	Body weight (g) after 21 days	Weight Difference (g)
I	5	22 %	1.506	1.438	− 68
II	6	35 %	1.232	1.430	+ 198
III	6	45 %	1.328	1.518	+ 190

* Plasma K and Na were determined by Prof. A. B. Ulhôa Cintra.

TABLE II

Food and caloric consumption and fecal caloric value of cats on purified diet with 35 % casein (100 g of dried ration = 480 cal.)

	Growing	Adults
Nº of cats	17	6
Period of observation	12 weeks	5 weeks
Average initial body weight	700 g	3.197 g
Average weekly gains	73.9 g	35.0 g
Average daily food intake per cat	38.1 g	65.6 g
Average daily food intake/kg body weight	33.1 g	19.8 g
Daily caloric consumption/cat	179.7 cal.	315.5 cal.
Daily caloric consumption/kg body weight	158.7 cal.	94.4 cal.
Daily fecal caloric value/cat	—	10.4 cal.

growing cats lose weight when placed on a cooked vegetable ration composed of equal parts by weight of compressed oats, dried beans, polished rice, dried split peas, and peanut meal, supplemented with 10 % peanut oil, 4 % bone ash, 1 % sodium chloride, and a complete vitamin supplement as given to the animals on purified ration: Vitamin B_{12}, liver extract* (1 ml), methionine (2 g daily), DL tryptophane (1.8 g daily), lysine (1.0 g daily) and cystine (1.0 g daily), did not produce weight response. when given separately. However, Dickson and Scott ([14, 15]) also observed that, on a mixed natural diet, with fish and liver as principal protein sources, at least 30 % of protein was necessary to support growth on young cats.

It has been observed (Carvalho da Silva) ([8]), that the cat grows faster on a stock ration than on purified diet with 35 % casein. Tentatives to improve the growth rate on purified ration by supplementing with raw beef liver, raw sardine, brewers yeast, were ineffective, although occasional growth responses could be observed during the periods of supplementation. In another experiment, 8 male cats about 10 weeks old, average weight 674 g (590 to 755) were maintained on purified ration during 160 days. After this period, the four animals with the least weight gains (average weight 1 280 g) received 0.2 ml of liver extract* daily, i. musc., during 21 days; the other cats (average weight 1 852 g) were kept as controls Thirty five days after the beginning of the supplementation period, the treated animals exhibited an average weight of 1 532 g and the control group 2 257 g. Although the growth rate of the supplemented group was improved, it did not reach the growth rate of the control animals and was still very far from the performances on stock rations (average weight for males after 28 weeks on experiment, 3 190 g, Carvalho da Silva) ([8]).

b) *Caloric consumption and fecal caloric value on purified diet.* The food intake of growing cats on purified diet was measured during 12 weeks on 17 animals caged in groups of two or three. Identical observations were carried

* Reticulogen "Lilly".

TABLE III

Chemical and physiological data on adult cats maintained on stock and purified ration

	STOCK RATION		PURIFIED RATION	
	Number of animals	Average values and range	Number of animals	Average values and range
Glucose, mg % (whole blood) ..... +	4	81 (77 — 86)	19	94 (67 — 148)
Plasma proteins, g %	6	6.1 (5.0 — 7.2)	6	6.1 (4.8 — 8.4)
Urea, mg % (whole blood)	5	62 (52 — 71)	6	59 (49 — 80)
Nonprotein N, mg % (whole blood)	6	42 (37 — 49)	6	43 (36 — 57)
Calcium, mg % (plasma)	6	10.8 (8.9 — 13.0)	4	10.3 (9.4 — 11.0)
Inorg. phosp., mg % (plasma)	6	6.2 (5.7 — 7.4)	6	4.4 (3.7 — 4.9)
Chloride, mg % (plasma)	5	408 (406 — 409)	4	441 (430 — 489)
Chloride, mg % (red cells)	3	273 (249 — 298)	3	319 (291 — 367)
Total Cholesterol, mg % (plasma)	5	94 (70 — 147)	3	87 (81 — 97)
Potassium, mg % (plasma)	—	— —	7	16.6 (14.1 — 23.8)
Sodium, mg % (plasma)	—	— —	7	354.2 (320 — 382)
Ascorbic acid, mg % (red cells)	7	0.27 (0 — 0.83)	5	0.49 (0.1 — 1.2)
Ascorbic acid, mg % (plasma)	7	0.63 (0.27 — 0.79)	5	0.93 (0.43 — 1.3)
DPN, mg % (red cells)	11	7.2 (5.1 — 10.0)	7	8.0 (5.6 — 10.3)
Pyruvic acid, mg % (whole blood)	—	— —	13	1.6 (0.8 — 2.5)
Lactic acid, mg % (whole blood)	—	— —	12	16.1 (4.3 — 28.6)
Iron, γ % (plasma)	—	— —	10	106 (50 — 200)
Adrenal ascorbic acid, mg %	6	79 (70 — 98)	5	70 (36 — 106)
Total liver fat, g %	6	5.7 (4.7 — 6.1)	6	14.3 (5.1 — 27.4)
Total fecal fat, g/day	—	— —	4	0.77 (0.53 — 0.89)
Total plasma volume, ml/100 g of body weight ...	—	— —	11	3.88 (3.0 — 4.5)
Total blood volume, ml/100 g of body weight	—	— —	11	6.96 (5.3 — 7.8)
Total body water, ml/100 g of body weight	—	— —	11	66.2 (58.4 — 74.4)

on 6 males caged individually, during five weeks. In this last group, the feces of each animal were collected daily and accumulated in the deep freezer (−10° C). The caloric consumption was calculated from the composition of the diet. The caloric value of the feces was determined on the pooled samples of each animal, on the calorimetric bomb, after careful homogeneization and drying. The results are summarised on table II.

Observations on the food consumption of cats specially for growing animals on purified diets must be conducted during long periods in view of the large daily variation. The amount of food necessary to maintain weight has not been determined with precision; on preliminary experiments, adult cats were able to maintain their weight after an initial loss of 100 to 200 g, when receiving 36 g of purified diet daily (aproximately 14 g/kg of body weight).

c) *Biochemical data, blood and plasma volume, total body water.* These results are summarised in table III. Blood was obtained from the saphena magna without anesthesia, or from the heart after anesthesia with nembutal intravenously (40 mg/kg of body weight). For adrenal ascorbic acid and liver fat determinations, the animals were anesthetised, bled at the heart, the abdominal cavity opened and the organs retired and wiped with filter paper; liver was kept in the deep freezer until used and the adrenals were immidiately homogenized (Potter and Elvehjem) ([25]) with metaphosphoric acid and assayed for ascorbic acid.

d) *Weight of the organs.* The weight of different organs and its relation to the body weight on adult cats maintained on purified ration are presented in table IV. The organs were removed after anesthesia with intra-venous nembutal and bleeding at the heart, wiped with filter paper and weight on triple beam or torsion scale. As the data on stock ration ade uncomplete and in smaller number, they are not presented. However, the relationship between organ weight and body weight were identical to the values observed on purified ration, with two exceptions: higher values for heart (average 0.30 % of body weight, range 0.34 to 4.47 on 7 cats) and lower values for liver.

Discussion

Most of the results obtained are identical for adult cats raised on stock and on purified diets. Two important differences however were observed: lower values for inorganic blood phosphorous and fatty livers on the animals on purified ration.

The lower values for inorganic phosphorous with normal calcium levels suggest a low vitamin D intake. Although the animals on purified ration received 2 ml of cod liver oil on alternate days, this was a commercial wholesale product without specifications for its vitamin D content.

The presence of fatty livers on animals receiving 35 % casein, and 0.3 g of choline and 0.03 g of inositol on alternate days indicated that the requirements of the cat for lipotropic factors should be investigated.

As the vitamin supplement used did not contain ascorbic acid the presence of normal values of this vitamin on blood and adrenals of animals on purified ration indicates a synthesis by the tissues.

Summary

Growing cats were not able to maintain their weight on 22 % casein but

TABLE IV

Weight of organs of adult cats on purified ration (6 males and 8 females)

Organ	MALES		FEMALES	
	Weight in g Average values and range	As percent of body weight Average values and range	Weight in g Average values and range	As percent of body weight Average values and range
Liver	88.5 (68 — 145)	2.95 (2.26 — 3.87)	67.6 (54 — 111.5)	2.65 (2.25 — 3.91)
Heart	9.75 (7.5 — 11.3)	0.33 (0.30 — 0.36)	8.1 (6 — 11.9)	0.34 (0.30 — 0.42)
Kidneys	20.2 (14.5 — 28.8)	0.69 (0.39 — 1.05)	16.6 (11.3 — 22.6)	0.68 (0.58 — 0.89)
Pancreas	7.7 (5.7 — 9.6)	0.28 (0.21 — 0.32)	7.8 (6.0 — 9.8)	0.29 (0.27 — 0.34)
Encephalon	24.7 (22.4 — 28)	0.89 (0.81 — 1.01)	25.7 (23.5 — 29)	0.98 (0.73 — 1.07)
Spleen	5.8 (3.1 — 9.7)	0.201 (0.08 — 0.309)	4.8 (2.8 — 8)	0.202 (0.100 — 0.340)
Stomach	20.0 (13.3 — 27.0)	0.72 (0.48 — 0.93)	19.7 (15.5 — 26)	0.68 (0.48 — 1.08)
Small intestine ...	81.6 (67 — 95.3)	2.83 (2.35 — 3.37)	78.1 (70 — 94.8)	2.9 (2.29 — 3.33)
Colon	19.1 (16.1 — 22)	0.70 (0.50 — 0.85)	18 (10 — 26)	0.66 (0.42 — 0.81)
Thyroid	0.195 (0.168 — 0.214)	0.0070 (0.0052 — 0.0081)	0.221 (0.141 — 0.264)	0.0083 (0.0062 — 0.0110)
Hypophysis	0.035 (0.022 — 0.038)	0.0012 (0.0009 — 0.0015)	0.028 (0.016 — 0.042)	0.0012 (0.0007 — 0.0015)
Adrenals	0.372 (0.290 — 0.465)	0.012 (0.011 — 0.014)	0.345 (0.167 — 0.525)	0.013 (0.007 — 0.017)
Testis	2.47 (1.2 — 3.5)	0.81 (0.47 — 1.09)	— —)	— —)
Uterum	— —	— —	2.35 (0.8 — 5.0)	0.93 (0.15 — 2.17)
Ovaries	— —	— —	0.489 (0.200 — 0.890)	0.20 (0.07 — 0.31)

identical growth rates were obtained on 35 or 45 %. Vegetable rations did not promote growth unless suplemented by casein or egg white. Occasional supplementation of the purified ration by beef liver, raw sardine and brewers yeast did not improve the growth rate on purified rations with 35 % casein; 0.2 ml Reticulogen daily for 21 days, produced a slight but not impressive improvement on growth.

Data are supplied on: average food intake, blood glucose, plasmatic proteins, blood urea, blood nonprotein N, plasmatic calcium and inorganic phosphorous, plasma and red cells chloride, blood cholesterol, plasma sodium and potassium, red cell, plasma and adrenal ascorbic acid, red cells DPN, plasmatic iron, blood pyruvate and lactate, total liver and fecal fat, plasma volume, blood volume, total body water and organs weight.

Low inorganic blood phosphorous with normal blood calcium and fatty livers were observed on purified ration.

Resumo

O nível de 22 % de caseina não é suficiente para manter o crescimento de gatos jovens. Com 35 % au 45 % o ritmo de crescimento é satisfatório, e egual para estes dois níveis. Embora o crescimento em 35 % de caseina seja inferior ao que se obtém em dieta natural, ele não é melhorado com suplementos de fígado crú, de sardinha crua, ou de levadura de cerveja. Uma ligeire melhora foi observada com 0.2 ml de Reticulogeno i.m. por dia, durante 20 dias. Em rações vegetais só se observou crescimento quando se incluiu caseina ou clara de ovo sêca.

Apresentam-se dados sobre: consumo de alimento, peso de orgãos, volume de agua total, volume total de sangue e volume plasmático, gordura hepática, gordura fecal, vlaor calórico fecal, ácido ascórbico em suprarenal, e alguns níveis bioquímicos em plasma (proteinas, cálcio, cloretos, potássia, sódio, ácido ascórbico, ferro, fósforo inorgânico), em glóbulos vermelhos (DPN, ácido ascórbico, cloretos) e em sangue (glicose, urea, nitrogênio não proteico, lactato, piruvato e colesterol).

Em dietas purificadas completas os animais apresentam um apreciável aumento da gordura hepática total.

References

(1) Albritton, E. C.: *Standard values in blood.* W. B. Saunders Company, Philadelphia and London, 1952.

(2) Albritton, E. C.: *Standard values in nutrition and metabolism.* W. B. Saunders Company, Philadelphia and London, 1954.

(3) Barker, S. B., and Summerson, W. H.: The colorimetric determination of lactic acid in biological material. *J. biol. Chem.*, 1941, *138*, 535.

(4) Bodansky, A.: Phosphatase studies. I. The determination of inorganic phosphate. Beer's law and interfering substances in the Kuttner-Lichtenstein method. *J. biol. Chem.*, 1932, *99*, 197.

(5) Brodie, B. B.: *Measurement of total body water. Methods in Medical Research*, vol. IV. The Year Book Publishers Inc., Chicago, 1951.

(6) Bueding, E., and Wortis, H.: The stabilization and determination of pyruvic acid in the blood. *J. biol. Chem.*, 1940, *133*, 585.

(7) Carvalho Da Silva, A.: The domestic cat as a laboratory animal for experimental nutrition studies. I. General considerations care and feeding of the animals. *Acta physiol. latinoamer.*, 1950 a, *1*, 20.

(8) CARVALHO DA SILVA, A.: The domestic cat as a laboratory animal for experimental nutrition studies. II. Comparative growth rate and hematology on stock and purified ration. *Ibid.*, 1950 b, *1*, 26.
(9) CARVALHO DA SILVA, A., FRIED, R., and DE ANGELIS, R. C.: The domestic cat at a laboratory animal for experimental nutrition studies. III. Niacin requirements and tryptophan metabolism. *J. Nutrition*, 1952, *46*, 399.
(10) CARVALHO DA SILVA, A., DE ANGELIS, R. C., PONTES, M. A., and GUERIOS, M. F. M.: The domestic cat as a laboratory animal for experimental nutrition studies. IV. Folic acid deficiency. *J. Nutrition*, 1955, *56*, 199.
(11) CARVALHO DA SILVA, A., and PONTES, M. A.: DPN, agua total e volemia em carência de acido nicotínico no gato. *Revista do Hospital das Clínicas*, 1957, *12*, 95.
(12) CAVALCANTI, J. A., and SONNLEITHNER, J. N. V.: Novo micrométodo de dosagem de uréa no sangue. *Brazil Médico*, 1933, *7*, 115.
(13) DAWSON, A. B.: The domestic cat in "The care and breeding of laboratory animals", edited by E. J. Farris; John Wiley & Sons, Inc., New York. Chapman & Hall, Limited, London, 1950.
(14) DICKINSON, C. D., and SCOTT, P. P.: Nutrition of the cat. I. A pratical stock diet supporting growth and reproduction. *Brit. J. Nutrition*, 1956, *10*, 304.
(15) DICKINSON, C. D. and SCOTT, P. P.: Nutrition of the cat. II. Protein requirements for grocoth of weanling kittens and young cats maintained on a mixed diet. *Brit. J. Nutrition*, 1956, *10*, 311.
(16) EISENMAN, A. J.: A note on the Van Slyke method for the determination of chlorides in blood and tissue. *J. biol. Chem.*, 1929, *02*, 411.
(17) FOLIN, O., and SVEDBERG, A.: Micromethods for the determination of non protein nitrogen, urea, uric acid and sugar in unlaked blood. *J. biol. Chem.*, 1930, *88*, 77
(18) FOLIN, O., and WU, H.: A system of blood analysis. *J. biol. Chem.*, 1919, *38*, 81
(19) GRIGAUT, A.: Dosage colorimétrique de la cholesterine dans l'organisme. *C. R. Soc. Biol.*, 1910, *118*, 827.
(20) HALD, P. M.: Determinations with flame photometer. *Methods in Medical Research*. The Year Book Publishers Inc., Chicago, 1951, *4*, 79.
(21) HALVERSON, J. D., and BERGEIM, O.: The determination of small amounts of calcium,
(22) HAWK, P. B., OSER, B. L. and SUMMERSON, W. H.: *Practical Physiological Chemistry*. The Blakiston Company, Philadelphia and Toronto. 12th Ed., 1949.
(23) LEVITAS, N.; ROBINSON, J.; ROSEN, F.; HUFF, J. W., and PERLZWEIG, W. A.: The fluorescent condensation product of N^1-methylnicotinamide and acetone. III. A rapid fluorometric method for the determination of total pyridine nucleotides in the red blood cells. *J. biol. Chem.*, 1947, *167*, 169.
(24) PHILLIPS, R. H., and HART, E. B.: The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. *J. biol. Chem.*, 1935, *109*, 657.
(25) POTTER, J. H., and ELVEHJEM, C. A.: A modified method for the study of tissue oxidations. *J. biol. Chem.*, 1936, *114*, 495.
(26) ROE, J. H., and KUETHER, C. A.: The determination of ascorbic acid in whole blood and urine through the 2,-4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J. biol. Chem.*, 1943, *147*, 399.
(27) TEIXEIRA MENDES, F., and GERMECK, O. A.: Determinação quantitativa do ferro plasmático. *Hospital*, 1950, *37*, 129.
(28) VAN DE KAMER, J. H., BOKKEL HUININK, H. B., and WEYERS, H. A.: Rapid method for the determination of fat in feces. *J. biol. Chem.*, 1949, *177*, 347.

ACCIÓN DE LAS INYECCIONES DE INSULINA SIMPLE PRACTICADAS EN LA VENA PORTA, SOBRE LA GLUCEMIA DE PERROS DIABÉTICOS ALOXÁNICOS

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SE HA PUBLICADO recientemente que la insulina pierde su efecto hipoglucemiante cuando se inyecta en la vena porta de perros diabéticos aloxánicos (1), es decir, cuando aquella hormona atraviesa el hígado, para alcanzar la circulación general. El autor del mencionado trabajo coloca un tubo en T, de goma, en la vena porta, lo que permite a voluntad introducir la insulina en el torrente circulatorio del perro por aquella vena: utilizó cantidades de 2, 5, 10 y 20 unidades de insulina regular de la casa Lilly, en perros que pesaban desde 15 hasta 25 kilogramos, pero el autor no expresa la proporción de insulina por kilogramo de peso inyectada en cada caso.

Hemos repetido estas experiencias con el afán de confirmarlas. Variamos un tanto la técnica, pues la colocación del tubo en T en la vena porta nos pareció un procedimiento engorroso y no indispensable, sin contar con la posible coagulación intravascular. Procedimos, pues, a inyectar la insulina simple en la vena porta, usando una jeringuilla bien calibrada, bajo anestesia y laparotomía previa del animal. En cada caso valoramos exactamente la cantidad de insulina inyectada, por unidad de peso del animal.

Utilizamos doce perros normales, doce perros diabéticos aloxánicos. dos perros diabéticos espontáneos, y dos perros aloxanizados que no se hicieron diabéticos. En cada caso se valoraron antes de la inyección de insulina, a los 10 minutos y a los 30 minutos después de la inyección, la glucidemia (2) (glúcidos reductores) y la levulosemia (3). La cifra de glucosa verdadera se obtiene por diferencia entre la glucidemia y la levulosemia.

Experiencias en perros normales

En perros normales nembutalizados se inyectó insulina simple en la vena porta. Comenzamos por 3 unidades de insulina simple por Kg de peso del animal, y la dosis fué gradualmente reduciéndose en los perros sucesivos, hasta llegar a 0.44 unidad por Kg. Se utilizaron las insulinas simples de las marcas

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TABLA I

PERROS NORMALES

Perro	Sexo	Peso en kg	Unidades de insulina inyectadas en la porta	Marca de la insulina	Unidades de insulina inyectadas en la porta por kg de peso	GLUCEMIA en mg × 100 ml: Antes de la inyección de insulina	GLUCEMIA en mg × 100 ml: A los 10 minutos de la inyección de insulina	GLUCEMIA en mg × 100 ml: A los 30 minutos de la inyección de insulina	LEVULOSEMIA en mg × 100 ml: Antes de la inyección de insulina	LEVULOSEMIA en mg × 100 ml: A los 10 minutos de la inyección de insulina	LEVULOSEMIA en mg × 100 ml: A los 30 minutos de la inyección de insulina
1	M	13.18	40	Lilly	3.0	81	77	26	4.2	2.8	2.7
2	M	6.82	20	Organon	2.90	55	42	28	3.0	2.6	2.2
3	M	7.72	20	Organon	2.60	57	35	27	3.3	4.6	3.4
4	H	18.12	40	Lilly	2.20	80	65	38	4.6	3.7	3.9
5	M	5.0	10	Novo	2.00	87	66	49	3.2	3.1	3.1
6	M	4.55	8	Lilly	1.76	82	66	34	3.3	3.1	3.4
7	M	9.10	15	Lilly	1.66	55	74	56	7.5	11.0	13.0
8	M	14.55	20	Lilly	1.374	75	75	39	4.0	3.8	3.5
9	M	14.55	20	Lilly	1.374	54	69	28	3.7	2.4	2.2
10	M	6.36	6	Novo	0.95	80	80	53	1.8	1.6	1.5
11	M	10.9	8	Novo	0.74	67	51	30	4.2	3.6	3.6
12	M	11.36	5	Novo	0.44	56	30	31	3.7	3.7	3.4

Lilly, Novo y Organón. En todos los casos, la respuesta, a los 30 minutos, fué de franca *hipoglucemia*, precedida, a veces, a los 10 minutos, de *hiperglucemia*. En el cuadro I aparecen tabulados los datos experimentales.

TABLA II

PERROS DIABÉTICOS ALOXÁNICOS

Perro	Sexo	Peso en kg	Unidades de insulina inyectadas en la porta	Marca de la insulina	Unidades de insulina inyectadas en la porta por kg de peso	GLUCEMIA en mg × 100 ml: Antes de la inyección de insulina	GLUCEMIA en mg × 100 ml: A los 10 minutos de la inyección de insulina	GLUCEMIA en mg × 100 ml: A los 30 minutos de la inyección de insulina	LUVELOSEMIA en mg × 100 ml: Antes de la inyección de insulina	LUVELOSEMIA en mg × 100 ml: A los 10 minutos de la inyección de insulina	LUVELOSEMIA en mg × 100 ml: A los 30 minutos de la inyección de insulina
13	M	7.72	20	Lilly	2.6	194	212	100	5.7	4.8	4.8
14	H	8.64	20	Organon	2.32	117	117	112	4.4	4.4	4.4
15	M	10.45	20	Lilly	1.92	675	668	632	23.1	23.5	24.4
16	M	12.3	20	Lilly	1.63	520	560	388	11.1	10.2	8.4
17	M	12.70	20	Lilly	1.58	284	210	82	4.2	4.4	3.6
18	M	20.80	30	Lilly	1.44	410	355	345	7.3	7.3	6.4
19	M	7.27	8	Novo	1.1	105	79	60	8.0	6.2	5.5
20	M	7.72	8	Novo	1.04	130	82	49	8.3	8.8	7.7
21	H	7.3	6	Novo	0.82	138	130	90	3.3	3.0	3.0
22	M	7.72	3	Novo	0.39	220	156	71	4.9	4.6	3.6
23	M	10.0	2.5	Novo	0.25	117	69	30	4.9	4.4	4.2
24	M	10.9	1.5	Novo	0.14	318	284	275	6.1	5.5	5.5

TABLA III

PERROS DIABÉTICOS ESPONTÁNEOS

Perro	Sexo	Peso en kg	Unidades de insulina inyectadas en la porta	Marca de la insulina	Unidades de insulina inyectadas en la porta por kg de peso	GLUCEMIA en mg × 100 ml: Antes de la inyección de insulina	GLUCEMIA: A los 10 minutos de la inyección de insulina	GLUCEMIA: A los 30 minutos de la inyección de insulina	LEVULOSEMIA en mg × 100 ml: Antes de la inyección de insulina	LEVULOSEMIA: A los 10 minutos de la inyección de insulina	LEVULOSEMIA: A los 30 minutos de la inyección de insulina
25	M	5.45	20	Lilly	3.67	102	94	78	3.0	2.9	3.0
26	H	3.18	6	Lilly	1.89	143	117	75	4.3	3.6	3.6

Experiencias en perros diabéticos aloxánicos

En perros diabéticos aloxánicos nembutalizados, se inyectó insulina simple en la vena porta. Se comenzó por 2.60 unidades de insulina simple por Kg de peso del animal, y la dosis fué progresivamente reduciéndose en los perros sucesivos, hasta llegar a 0.14 unidad por Kg. Se utilizaron las insulinas simples de las casas Novo, Lilly y Organón, con una invariable respuesta *hipoglucémica*, a veces precedida de *hiperglucemia*. El cuadro 2 resume los datos experimentales. En este cuadro es preciso advertir que el perro ♯ 14 fué aloxanizado tres veces, porque las dos primeras no respondió al aloxán, y parece que su resistencia al aloxán se tradujo también en una resistencia a la insulina, pues claramente se ve que, en este caso particular, la hipoglucemia observada fué muy pequeña. La perra ♯ 21 estaba encinta.

Experiencias en otros perros

En dos perros diabéticos espontáneos y en otros dos perros aloxanizados que no se hicieron diabéticos, realizamos iguales experiencias con idénticos resultados. Las tablas 3 y 4 presentan los datos experimentales al respecto.

RESUMEN Y CONCLUSIONES:

Se estudia la acción de la insulina simple, inyectada por la vena porta a perros normales, diabéticos aloxánicos, diabéticos espontáneos y aloxanizados

TABLE IV

PERROS ALOXANIZADOS QUE NO SE HICIERON DIABÉTICOS

Perro	Sexo	Peso en kg	Unidades de insulina inyectadas en la porta	Marca de la insulina	Unidades de insulina inyectadas en la porta por kg de peso	GLUCEMIA en mg × 100 ml: Antes de la inyección de insulina	GLUCEMIA: A los 10 minutos de la inyección de insulina	GLUCEMIA: A los 30 minutos de la inyección de insulina	LEVULOSEMIA en mg × 100 ml: Antes de la inyección de insulina	LEVULOSEMIA: A los 10 minutos de la inyección de insulina	LEVULOSEMIA: A los 30 minutos de la inyección de insulina
27	M	7.27	30	Novo	4.13	90	68	37	3.5	2.6	2.6
28	H	7.27	4	Novo	0.55	65	29	25	4.6	4.6	4.6

no diabéticos. Los resultados obtenidos, trabajando en las condiciones experimentales señaladas, han sido los siguientes:

Primero: En cuanto a la *glucosa circulante,* se presentó siempre, tanto en los perros normales como en los diabéticos aloxánicos, en los diabéticos espontáneos y en los aloxanizados no diabéticos, una franca *hipoglucemia* a los 30 minutos, precedida en algunos casos de *hiperglucemia* en los 10 primeros minutos.

Segundo: En lo que se refiere a *levulosemia,* los hallazgos han sido muy variables. En los perros 1, 2, 5, 6, 8, 9, 10, 11, 12, 13, 16, 18, 19, 21, 22, 23, 24, 26 y 27, se aprecia un ligero descenso; en los perros 7 y 15, hay una elevación sostenida; en los perros 3, 17 y 20, una elevación inicial; en los perros 4 y 25, una caída inicial con elevación posterior; y en los perros 14 y 28, el nivel se mantiene prácticamente igual durante toda la prueba.

Summary

This report is concerned with the effects of injecting regular insulin in the portal vein of anesthetized dogs.

We used 12 alloxanized diabetic dogs; 12 normal dogs; two spontaneously diabetic dogs; and 2 non-diabetic alloxanized dogs.

The insulin injected varied from 0.44 to 3 units per Kg, in the alloxanized dogs; and from 0.14 to 2.60 units per Kg in the normal dogs.

In all cases the result was *hypoglycemia.*

The *levulemia* curves varied from case to case.

Referencias

(1) Jáuregui, Raúl H.: *Revista Medicina,* México. Tomo XXXVI, Año XXXVII, Nº 759. Págs. 503-516. Noviembre 10, 1956.

(2) Somogyi-Nelson: *J. biol. Chem.,* 1945, *160,* 62; 1944, *153,* 376.

(3) Higashi-Peters: *J. Lab. and clin. Med.,* 1950, *35,* 475.

ARGINASE: EFFECTS OF MECHANICAL AGITATION AND OF ULTRASONIC RADIATION

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In view of the widespread of ultrasonic radiation for various therapeutic purposes (neuralgic conditions, rheumatism, etc.) and the importance of arginine in metabolism, it is interesting to determine the effect of ultrasonic waves on arginase and consequently on arginine.

The purpose of these experiments was to determine the effects of ultrasonic waves (US) on arginase activity at different frequencies, energies and concentrations, in atmospheres af air, oxygen and hydrogen at intervals up to one hour, and delayed effects at intervals of one day or more up to at least 21 days; also the effect on pH, total nitrogen, viscosity, agglomeration, and related phenomena, and to find, if possible, a reasonable explanation of the observed phenomena. In connection with this it was thought desirable to study cuantitatively the effects of simple mechanical agitation, under parallel conditions.

Available US studies on enzymes and proteins are not so numerous as investigations on non-protein high polymers. The latter differ widely in their behavior from most proteins and are relatively much more regular and consistent in their reactions. Among the enzymes, studies have been reviewed by Grabar [1] on oxidase in fruit and milk, catalase, peroxidase, reductase and amylase, hyaluronidase, pepsin and cathepsin. In addition we have noted the following, most of them since the date of Grabar's review: cholinesterase [2], cytochrome *c* [3], pepsin [4], xanthinedehydrogenase [5], catalase [6], alkaline phosphatase [7], hyaluronidase [8], salivary amylase [9] and yeast invertase [10]. In general, all these have shown initial inhibition or activation, followed generally by definite inhibition depending on energy, frequency, duration of exposure, concentration, and other conditions not always specified with sufficient precision.

Materials. Arginase from calf liver highly purified by a procedure described elsewhere [11], containing a small amount of catalase and traces of other impurities, and free ions of Mn and Co, at pH 6-7, was used, at activities (AU) from 15 to 52 as determined by the method of Mohamed and Greenberg [12].

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Mechanical agitation

Method. The apparatus consists of a wooden base to one end of which is hinged a vertical plank which is given reciprocating motion by a variable-speed motor mounted on the base together with a rheostat. The vertical plank has clasps to hold glass tubes of the same diameter as the cups of the US apparatus, 2.8 cm, into which 16 cc of the arginase solution were placed for each experiment, this being the same amount as that used in the US experiments. The surface of the solution moved through an arc of 5.6 cm. Experiments were made at 40, 80 and 120 strokes per minute (s/m) and interrupted at 3, 5, 15, 30, 60 and 90 minutes for duplicate assays of 0.1 cc at each interruption, for the experiments in air. Tests in hydrogen were run for 60 minutes without interruption, and after having passed H_2 through the solution for 30 minutes in order to expel the air and other gases. The O_2 tests were made simply by bubbling the gas through the solution for 30 minutes without agitation. Temperature and pH (Beckman) were taken before and after each experiment.

For delayed effects, many runs of three consecutive tests from the same stock were kept together in test tubes at 6° and assayed together at various intervals, after centrifuging, at the same time with the initial stock which served as control.

Results. At 40 s/m there was practically no surface agitation, at 80 s/m agitation was moderate and at 120 s/m violent with much foaming, similar to that in high-energy US treatment but of course without the geyser. In air there was a slight activation at 3 minutes at 40 and 80 s/m, and no change at 120. In all cases this was followed by a loss of 5 %, 7 % and 8 % respectively at 30 minutes, and little if any loss thereafter. But while the activities in air at 60 minutes were 93 %, 93 % and 92 % at 40, 80 and 120 s/m respectively, the corresponding activities in H_2 were 98 %, 98 % and 99 %, while that of the solution through which O_2 had been passed was 87 %.

In all cases temperature remained practically constant, at 22°. pH: at less than 80 strokes there was no significant change of pH; at 80 and 120 strokes the average pH rose from 6.65 to 6.82, but recovered within a few days. Phosphate buffer at pH 6.8 showed no change after agitation for 120 minutes at 120 strokes.

Distilled water agitated for 120 minutes at 120 strokes was negative to tests for NO_2, NO_3, and H_2O_2.

In no instance was there any visible turbidity at 60 minutes, but there was later some slight sedimentation in solutions treated at 80 and 120 s/m.

Measurements of delayed effects showed a bewildering variety in which it was not possible to find any consistent correlation with s/m, initial concentration or atmosphere. In Table I are three fairly typical cases selected from more than 100. The striking feature in three of them is the partial recovery of activity after an interval of days, while one shows only inactivation. The one datum common to all is eventual inactivation.

Discussion. The explanation of these phenomena is to be sought in the three new factors in presence: thermal, mechanical, chemical. As there were no significant changes in temperature the thermal factor may be eliminated at once. Mechanical agitation, to be effective, must have been sufficiently violent to disrupt or distort the arginase molecule. This could occur only through the shock at the end of each stroke. At 40 and 80 s/m this shock is very mild; at 120 s/m it is stronger and more frequent, and may perhaps be

TABLE I

Delayed effects of shaking at 120 s/m; A and C in air, B and D in hydrogen, 100 % = activity at end of run

Days	PERCENTAGE OF ACTIVITY			
	A	B	C	D
3	69	91	76	87
7	94	80	44	69
14	75	90	84	56
21	58	74	71	50

operative to a very small extent. The really effective factor is suggested by the facts that (a) in an atmosphere of hydrogen the inactivation is almost negligible at 60 minutes; (b) in an oxygen atmosphere the activity at 60 minutes is reduced to 87 %, and (c) the activities at 60 minutes in air are 92 % and 93 %. The conclusion seems inevitable that the observed inactivation is due to oxidation, the chemical factor. It may be that the slight inactivation in hydrogen is due to small amounts of oxygen remaining in the solution, and/or to mechanical shock either negatively on the active arginase molecule or positively by aiding the activation of proarginase through adjunction of free Mn or Co ions.

No verifiable explanation of the erratic delayed effects, especially of the very frequent partial recoveries, is yet available.

Ultrasonic radiation

Methods. The US apparatus employed was the Siemens "Universal Sonostat", at a basic frequency of 0.8 megacycles (Mc) and at the third harmonic 2.4 Mc, and with a maximum of 52 watts delivered to the 10 cm^2 emitting surface, i.e. 5.8 w/cm^2. 16 cc of the arginase solution were placed in a glass cup of 35 cc volume, diameter 2.8 cm and height 6.0 cm with an electrolytic copper base 0.1 mm thick, at 5 cm from the applicator. Circulating water kept the temperature constant within 2 or 3 degrees.

The energies used were 1.2, 2.4, 3.6 and 4.8 w/cm^2, the frequencies 0.8 and 2.4 Mc. For the early experiments in air, the cup was covered with a perforated cap which eliminated spattering but still permitted surface agitation. This necessitated the above study of the effects of simple surface agitation, the results of which were used for correction when necessary. Later the cup was left open for the first 2 ½ to 3 minutes, which allowed the escape of gases before serious surface agitation set in, and then, in order to suppress the surface agitation, it was closed with a floating cork until the end of the experiment. Radiation was stopped at intervals for the taking of assay samples. In another series of tests the air was replaced by hydrogen and the cups sealed. In the oxygen tests the air was replaced by hydrogen and the cups sealed. In the oxygen tests the gas was bubbled through the solution for 60 minutes. Assays in air were made at regular intervals up to 60 minutes. In the hydrogen tests, the runs were for

30 minutes without interruption, and for comparison similar uninterrupted runs were made in air. pH (Beckman) and viscosity (Ostwald) were measured before and after each run. Temperature was measured before, during, and after each series.

Results. The results in air expressed in percentages of initial activity, are given in Fig. 1, and in H_2 in Fig. 2. The outstanding conclusion is that in air there is a progressive inactivation uninterrupted by even transient recoveries such as occur with ionizing X-rays ([13]).

The inactivation is faster and more irregular during the first 10 minutes than after. The floating cork reduced to some extent the access of air to the solution and may have contributed to the retardation of the rate of inactivation evident after 10-15 minutes: but as the same retardation occurs in an atmosphere of hydrogen, the effect of closure appears to be negligible. Several series of tests were made at intervals of one minute for four minutes. At 0.8 Mc—4.8 w/cm² the results were 96 %, 94 %, 90 % and 91 %. During the first few minutes the inactivation in both series was much more rapid, and the differences between observations reflected a state of relatively greater disorder.

The experiments in an atmosphere of hydrogen were run at 0.8 Mc and

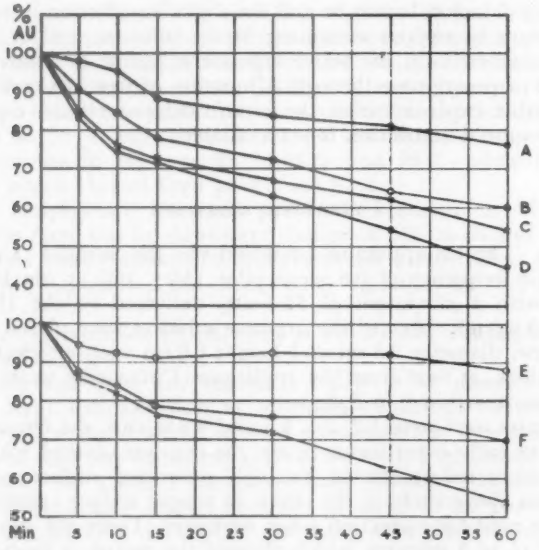

FIG. 1.—*Inactivation of arginase by ultrasonic waves in air. The abscissa is time in minutes; the ordinate is activity in percentage of initial activity. The curves are as follows:*

Curve	*Mc-w/cm²*	*Curve*	*Mc-w/cm²*
A	*0.8-1.2*	*E*	*2.4-1.2*
B	*0.8-2.4*	*F*	*2.4-2.4*
C	*0.8-3.6*	*G*	*2.4-4.8*
D	*0.8-4.8*		

1.2, 2.4, 3.6 and 4.8 w/cm² for 30 minutes without interruption. The H_2 series gave 96 %, 83 %, 84 % and 77 % respectively, and the corresponding air series 92 %, 80 %, 74 % and 67 %. That is: at each energy level the inactivation was considerably greater in air than in hydrogen.

Influence of Energy and Frequency. Comparison of results at varying energies and constant frequency shows, as would be expected, accelerated rates

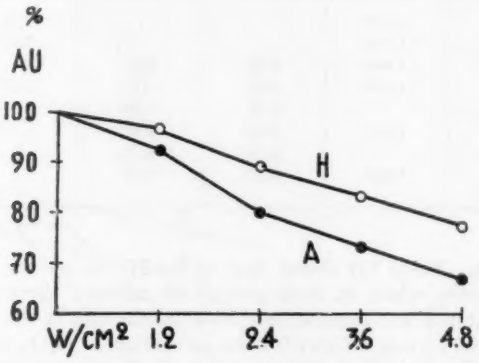

FIG. 2. — *Effect on arginase activity of 30 minutes US irradiation at 0.8 Mc, in air and in hydrogen.*
A ... in air.
H ... in hydrogen.

of inactivation with increasing energy. On the other hand, at corresponding energies the frequency 2.4 Mc inactivates less rapidly than the frequency 0.8 Mc., as shown in Fig. 1.

Influence of Concentration. As shown in Table II, inactivation is progressively more rapid, at least after the first few minutes, as the initial activity is higher.

TABLE II

Influence of concentration on inactivation of arginase by ultrasonics, in air

AU₀	1'	2'	3'	4'	5'	10'	30'	60'
15	96	97	95	94	95	87	71	57
39	99	92	90	83	84	80	61	49
53	95	96	95	89	82	74	60	40

Effect on Viscosity. As shown in Table III, at the lower intensity 0.8 - 1.2 there was no change in viscosity: at all other combinations the viscosity was lowered. Turbidity was increased very slightly, usually observable only in the microscope dark field. In no case was there precipitation.

TABLE III

Effect of US irradiation on viscosity and pH of arginase in air

Mc-w/cm²	VISCOSITY		pH IN AIR		pH IN H_2	
	0 min	60 min	0 min	60 min	0 min	60 min
0.8-1.2	1.050	1.050			7.35	7.35
0.8-2.4	1.055	1.042			7.36	7.35
0.8-3.6	1.060	1.050	6.07	6.12		
0.8-4.8	1.091	1.075	6.07	6.17	7.01	7.01
0.8-4.8			6.83	7.00		
2.4-1.2	1.046	1.042	6.84	6.62		
2.4-2.4			6.76	6.52		
2.4-4.8	1.077	1.068	6.12	6.07		

Effect on pH. Table III shows that at 0.8 Mc in air the pH was always higher at 60 minutes, while in hydrogen at 60 minutes there was no change. At 2.4 Mc in air the pH was considerably lower at the end of the 60 minute runs.

Other Chemical Effects. Tests for the formation of H_2O_2 with catalase and with $K_2Cr_2O_7$ - ether gave negative results in both the air and the hydrogen series. This however does not prove that none was formed. Small amounts would have been destroyed by the catalase remaining in the solution and also by oxidation-reduction reactions with the proteins and with the Mn^{++} and Co^{++} ions present. Tests for total N showed no changue at 2.4-2.4 and 2.4-4.8 in air with initial N at 0.52 mg/cc and 0.49 mg/cc respectively. Seven solutions were deproteinized and spot tested for NO_2 and NO_3 all with negative results. In the test for copper, distilled water and phosphate buffer pH 7.2 were irradiated for two hours at 0.8-4.8: no trace of Cu^{++} was found.

Reversibility. Attempts to restore activity in whole or in part with Mn and Co ions were unsuccessful.

Delayed Effects. Changes in activity following cessation of US irradiation and tested at intervals in 35 solutions were different for each solution and duration of exposure, whether in air on in hydrogen. Fig. 3 charts five averages drawn from 12 solutions covering a wide variety of initial conditions. From these curves four conclusions may be suggested: 1/ after irradiation inactivation continues, though at a lower rate; 2/ in the meantime the enzyme shows frequent partial and transient recoveries; 3/ there is no apparent correlation between the rates of delayed inactivation and the initial conditions, with the following exception; 4/ solutions irradiated in hydrogen inactivate further and more rapidly than the others, and with no recoveries.

These observations raised the question whether the deterioration was due to production of some toxic substance during irradiation such as occurs with X-rays ([13]). Completely inactivated US-irradiated arginase which had been kept for 6 months or more was added to untreated enzyme in the proportions 1/4, 1/2, 1/9 and 3/2 respectively, keeping the concentration of 1 cc of arginase constant by raising the whole to 3 cc. Activities were determined after 24 hours at 6°. The results: 99 %, 97 %, 97 % and 100 % respectively, must be considered prac-

tically negative. The post-irradiation inactivation is not due to any newly formed chemical product.

Discussion. What is the mechanism of the inactivation of arginase in solution by the application of US waves? There are four possibilities: 1/ thermal; 2/ chemical; 3/ mechanical; 4/ some combination of the these three.

Thermal action. In these experiments the temperature never rose more than 3° above the initial 18° to 20°. The increase was usually about 2.5 to 3°

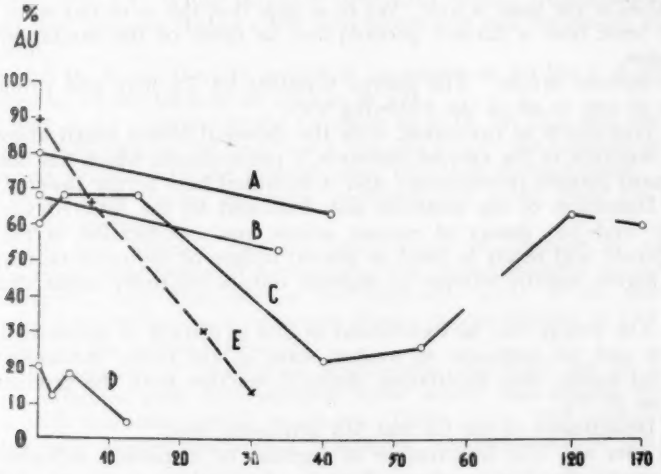

FIG. 3. — *Delayed effects of US irradiation on arginase activity. In five instances, after stoppage of irradiation. Abscissa: days elapsed. Ordinate: % activity; taking AU at end of irradiation as 100 %.*

Curve	*Mc-w/cm²*	*Minutes exposed*
A	*2.4-4.4*	*15 min. in air*
B	*2.4-5.2*	*120 min. in air*
C	*2.4-2.4*	*60 min. in air*
D	*2.4-5.2*	*120 min. in air*
E	*1.2-4.8*	*60 min. in hydrogen*

and occurred within the first three minutes, after which variation did not exceed 1°. Within this temperature range such variations have no appreciable effect on arginase activity [14].

Chemical action. The substances produced by US in aqueous solutions (leaving aside oxidations of simple organic and inorganic compounds) are reported to be $-NO_2$, $-NO_3$ and H_2O_2 [15, 16] and/or H_2O radicals due to cavitation [1, 17, 18]. If H_2O_2, an arginase inhibitor, were produced in these solutions it would have been destroyed at once by the small amounts of catalase remaining in them. A small amount of NH_3 could be detected in the gases liberated during the first 2 or 3 minutes, and tests showed not more than doubtful traces of other nitrogen compounds; in any case they were certainly not sufficient to have a significant inhibitor effect. On the other hand the

very marked difference between the rates of inactivation in air and in hydrogen suggests that an oxidation of the enzyme has occurred, due to the production of OH through the action of cavitation. Arginase presents many –H and –N bonds exposed to chemical reactions. The resulting changes amount to a structural alteration of the enzyme molecule rendering it inapt to combine effectively with the substrate.

If the theory that the degree of cavitation is lowered with increase in frequency (and chemical action thereby decreased) is correct [1, 19, 20], then we would expect inactivation to be less rapid at higher frequencies than at lower frequencies at the same w/cm². We have seen that this is in fact what happens, and we have here a further presumption in favor of the cavitation-chemical mechanism.

Mechanical action. The energy supplied by US may and probably does operate in any or all of the following ways:

1/ Production of cavitation, with the chemical effects noted above.

2/ Rupture of the enzyme molecule, a phenomenon which has been observed by many protein investigators, and is indicated here by the lowered viscosity.

3/ Distorsion of the molecule also indicated by the lowered viscosity: on the lock–and–key theory of enzyme action, any modification of the form of the molecule will result in total or partial failure of the catalytic reaction. In such a highly specific enzyme as arginase only a relatively slight distortion is needed.

4/ The energy may be insufficient at first to disrupt or seriously distort the molecule and yet sufficient to weaken some of the many intramolecular and peripheral bonds, thus facilitating chemical reaction with the products due to cavitation [21, 22].

5/ Detachment of the Co and Mn activating ions.

To sum up: The inactivation of arginase by ultrasonic radiation appears to be brought about in two ways. First, by chemical reaction with the products of cavitation, involving oxidation; and second, by mechanical alteration of the structure of the molecule. While the two mechanisms operate jointly and additively, the chemical mechanism appears to predominate in an atmosphere of O_2 while in hydrogen the mechanical action may be more important, although even in the absence of O_2 oxydation may be produced by cavitation and may therefore be an additional chemical mechanism [23].

Delayed effects. These considerations, while they account adequately for the phenomena observed during the process of irradiation, are not sufficient to explain the widely scattered results shown in the delayed effects. The continued inactivation is not due to any toxic substance; the precise sequence and extent of these phenomena seem in experience to be unpredictable and to vary with each individual. Present attempts at explanation would involve much speculation. Innumerable similar observations are reported in the biological and biochemical literature, and their elucidation is an urgent need.

Summary

1. Mechanical agitation in air produces a slight activation of arginase in the first 3 - 4 minutes, followed by progressive inactivation reaching 7 % - 8 % at 60 minutes. In an atmosphere of hydrogen there is no significant change in activity. This indicates that the inactivation in air is due to oxidation by the gas dissolved in the solution and in the air at the agitated surface.

2. Ultrasonic irradiation at frequencies 0.8 Mc and 2.4 Mc and energies of 1.2, 2.4, 3.6 and 4.8 watts/cm^2 inactivates arginase in air at an approximately exponential rate, more rapidly at first and more slowly thereafter, up to a loss in air of 55 % at 0.8 Mc and 46 % at 4.8 w/cm and 60 minutes. At the same intensity the loss in air at 30 minutes is 37 % and in an atmosphere of hydrogen, 23 %.

3. The rate of inactivation increases with increasing energy and concentration and decreases with increasing frequency.

4. Viscosity is lowered at all frequency-energy combinations in air, except at the lowest, 0.8 - 1.2. While turbidity developed during irradiation is slight, it increases and becomes visible a few days afterwards and eventually results in precipitation.

5. At 0.8 Mc in air the pH is slightly increased; at 2.4 Mc it is decreased. In hydrogen at 0.8 Mc there is no change in pH.

6. There is no evidence of the presence of H_2O_2 or NO_3 and only slight indication of NH_3 during the first 2 or 3 minutes in air. No arginase-inhibiting substance was produced in the solution after cessation of irradiation.

7. Temperature during exposure increased not more than 2°–3° from initial room temperature.

8. These data indicate thet the inactivation of arginase by ultrasonic irradiation in an atmosphere of hydrogen is due almost entirely to mechanical action—disruption and distortion of the molecule, while in air there is in addition the chemical action of oxidation due to the production of OH radicals by cavitation in the aqueous solution.

9. After cessation of irradiation inactivation continues but at unpredictable rates, different with each solution, more slowly than during irradiation and often with transient partial recoveries. There is no clear correlation between subsequent history and initial intensities, concentrations or duration of exposures.

References

(1) Grabar, P.: *Adv. Biol. Med. Physics*, 1935, *3*, 191.
(2) Buonsanto, M. and A.: *Boll. soc. ital. biol. sper.*, 1953, *29*, 158.
(3) Haas, E.: *J. biol. Chem.*, 1943, *148*, 481.
(4) Naimark, G. M., Mosher, W. A.: *J. Acoust. Soc. Amer.*, 1953, *25*, 289.
(5) Robert, M. L., Nolla, N.: *Bull. soc. chim. biol.*, 1953, *35*, 1057.
(6) Amici, A.: *Boll. soc. ital. biol. sper.*, 1952, *28*, 1930.
(7) Vantaggi-Cozzari, L., Levi, F. A.: *Boll. soc. ital. biol. sper.*, 1953, *29*, 1723.
(8) Santamaría, L., Castellani, H., Levi, F. A.: *Enzymologia*, 1952, *15*, 285.
(9) Buonsanto, M.: *Boll. soc. ital. biol. sper.*, 1952, *28*, 950.
(10) Oparin, A. J., Bardinskuya, M. S., El Piner, I. E.: *Doblady Akad. Nauk S.S.S.R.*, 1954, *99*, 423. *Chem. Abs.*, *49*, 5542.
(11) Thompson, C. B.: *Am. J. Physiol.*, 1952, *170*, 731.
(14) Thompson, C. B.: *Arch. Soc. Biol. Montevideo*, 1950, *17*, 34.
(15) Akiya, S., Okni, A.: *J. Pharm. Soc. Japan*, 1947, *67*, 232.
(16) Mastagli, P., Mahoux, A. P.: *Comp. rend.*, 1947, *224*, 276.
(17) Haissinsyk, M., Prudhomm, R. O.: *J. chim. physique*, 1950, *47*, 925.
(18) Bellavita, F., Levi, F., A., Cagnoli, N.: *Ann. chim.* (Rome), 1955, *45*, 248.
(19) Noltingk, B. E., Nappiras, E. A.: *Proc. Physical Soc.* (London), 1950, *C3 B*, 674.
(20) Dognon, A., Simonot, J.: *J. chim. physique*, 1953, *50*, 94.
(21) Talmud, D. L.: *Chem. Zeitschr.* (Russian Zone ed.), 1948, *1*, 591.
(22) Pasynskii, A. G., Chernyak, R. S.: *Doblady Akad. Nauk S.S.S.R.*, 1951, *79*, 1001. *Chem. Abs.*, *49*, 12551.
(23) Prudhomme, R. O., Grabar, P.: *J. chim. physique*, 1949, *46*, 323.

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SANTIAGO DE CHILE

June 26, 1957

Combined effect of cardiac glycosides and low potassium on the isolated guinea pig heart and auricles. M. PENNA, A. ILLANES, F. RIVAS AND J. MARDONES. *(Instituto de Farmacología, Universidad de Chile).*

The effects produced by changes of the potassium concentration of the medium, either independently or associated with k-strophantoside, on the electrogram and the isotonic contraction of isolated guinea pig auricles and on the electrogram of isolated guinea pig heart, were studied.

The decrease of potassium concentration produced a constant increase of the amplitude of the contraction and arrhythmia in one fourth of the isolated auricles, and a constant enlargement of PQ interval and arrythmia in one third of the isolated hearts.

K-strophanthoside (0,25 mg) produced an inconstant increase of the amplitude of the contraction of isolated auricles and arrhythmia in half of the auricular and in about one third of the isolated hearts electrograms.

The effects of combined K-strophantoside and of low potassium on the rhythm of auricles and hearts are synergistic. (Arrhythmia in ¾ of the auricles and in all of the hearts). However, the effect on the amplitude of the contraction of isolated auricles was not higher than the one obtained with low potassium Tyrode in the absence of glycoside.

The effect of adrenaline and noradrenaline on the activity of denervated skeletal muscles. Antagonism between curare and adrenaline substances. J. V. LUCO AND P. SÁNCHEZ. *(Laboratorio de Neurofisiología Gabriela Gildemeister, Universidad de Chile).*

In a previous paper of this laboratory (Luco and Sánchez, Acta Physiol. Lat. Amer.: *6*: 171; 1956) it was reported that facial denervated muscles give a contractile response to adrenaline injections. They also observed that these muscles show "spontaneous" activity and that it is increased by adrenaline.

The present paper demonstrates that noradrenaline produces the same effect and that these muscles are even more sensitive to this drug.

The electrical activity was also studied: Adrenaline and noradrenaline produce an increase of the electrical activity of denervated muscles for a very short period. Then it decreases the activity and one or two minutes later a prolonged increase is observed. In many instances, the last stage is characterized by trains of synchronized spikes. The effect lasts for several minutes and it has a temporal course similar to the "spontaneous" contractile activity seen after adrenergic substances.

The contractile effect of curare (Mc Intyre, A. R.; King, E. E. and Dunn, A. L. J. Neurophysiol: *8*: 297; 1945) is accompanied by an increase of the electrical activity, followed by a decrease. Curare blocks the contractile response produced by adrenaline and noradrenaline.

July 9, 1957

Mechanism of action of the antidiuretic hormone. Studies of the effects of combined administration of acetolamide and pitressin. M. SAPAG AND M. CANESSA. *(Cátedra de Bioquímica Clínica, Escuela de Química y Farmacia, Universidad de Chile).*

Some aspects of the mechanism of action of antidiuretic hormones were evidenced studying simultaneously the osmolar clearance, the free-water clearance, free-water reabsorption and the isoosmotic reabsorption of water and solutes in normal subjects under the combined action of acetazolamide and Pitressin.

At the same time the electrolites, the acid base equilibria of urine and plasma, and the glomerular filtration rate of water and solutes were studied.

It was seen that pitressin promoted a parallel reabsorption of water and solutes, as shown specially by sodium, phosphates, bicarbonates and chlorides. The hormone did not influence the inhibition in the production of H^+ and NH_4 provoked by acetazolamide, nevertheless it reestablished $BHCO_3$ and B_2HPO_4 reabsorption. These findings suggest that Pitressin may act at the level of another enzymatic system of the distal tubule in a close relationship to the active substraction of solutes at this site.

Influence of oral administration of thyroid powder on the voluntary alcohol intake by rats. RAFAEL PRIETO, ANÍBAL VARELA AND JORGE MARDONES. *(Instituto de Farmacología, Instituto de Investigaciones sobre Alcoholismo, Universidad de Chile).*

Twenty one rats, 12 males and 9 females, from the "non drinking" substrain of the "Instituto de Educación Física of the Universidad de Chile" fed with stock diet and exhibiting a basal voluntary alcohol intake lower than 0,15 ml/100 g body weight/day of 95 % alcohol, were given the same diet containing 0,5 % of thyroid powder during three weeks. The alcohol intake increased from a basal level of $0,05 \pm 0,007$ to $0,22 \pm 0,06$ during the third week of thyroid treatment. This increase is significant at the 2 % level. After returning to basal diet the alcohol intake decreased rapidly to a level significantly different from the basal one.

Three female rats of the same stock exhibited a higher basal voluntary alcohol intake, namely 0,23, 0,25, and 0,66 ml/100 g body weight/day. During the second and third week of thyroid treatment, the voluntary alcohol intake increased to 0,27, 0,57 and 1,20 respectively.

All the 24 rats increased their fluid intake during thyroid treatment to about the double of the basal consumption, and after returning to the stock diet, they decreased it reaching the normal level on the second week.

The body weight decreased during thyroid treatment in $8,9 \pm 1,2$ %. These results on the changes in the alcohol intake are not in agreement with those reported by Richter (Endocrinology *59*: 472, 1956).

Comparative study of the effects of bled and phenylhydrazine treated rabbits' plasma on iron metabolism. G. HODGSON, I. ESKUCHE, D. YUDILEVICH, P. HERNÁNDEZ AND J. TOHÁ. *(Sección Biológica, Laboratorio de Física Nuclear Pura y Aplicada, Universidad de Chile).*

Plasma obtained from phenylhydrazine treated rabbits markedly increases plasma iron turnover. The supernatant obtained after boiling this plasma, conserves its effect on iron turnover.

Bled rabbit's plasma does not affect iron turnover, but has a clear cut lowering effect on

plasma iron. Dialyzed plasma of bled rabbits however, increases plasma iron turnover, while acetone extracts of bled rabbits' plasma, significantly depress plasma iron and plasma iron turnover.

The fraction of Fe^{59} appearing in the red cells at 24 and 48 hrs. is increased by normal plasma, bled rabbits' plasma, phenylhydrazine trated rabbits' plasma and is not a good index of the state of erythropoiesis.

Analysis of the effect of anemic rabbits' plasma on iron metabolism as studied with tracer doses of Fe^{59} gives valuable information on the factor controlling iron metabolism and presents a practical method for the bio-assay of the plasma erythropoietic factor "Hemopoietine".

July 23, 1957

Effects of the absence of calcium alone or associated to cardiac glycosides, histamine or epinephrine on isolated heart preparations. MARIO PENNA, ALEJANDRO ILLANES, RAFAEL VEGA AND JORGE MARDONES. *(Instituto de Farmacología, Escuela de Medicina, Universidad de Chile).*

The changes of the amplitude of contraction and of the electrogram of isolated guinea pig hearts and auricles produced by the absence of calcium in the medium were studied.

In both preparations the change of the medium for one without calcium stopped the contraction, but the electrogram persisted with unchanged frequency. In the isolated heart, QT time was enlarged. When the perfusion without calcium lasted longer than 3 minutes after the dissapearing of the contraction, the rhythm was always altered and in one case fibrillation appeared. The restoration of the calcium content of the medium in both preparations restored the amplitude of the contractions and the altered rhythm.

The addition of lanatoside C ($1 - 1.5 \times 10^{-5}$ mM/l) to isolated auricles bathed in Tyrode solution without calcium did not restore the amplitude of the contraction. In the same conditions, histamine (0,5 – 1 mM/l) partially (16.9 ± 8 %) and epinephrine (1×10^{-8} mM/l) almost completely restored (72 ± 23 %) the amplitude of the contraction. The last two substances also showed a positive chronotropic action.

Action of syntocinon on the excretion of water, sodium and potassium in rats treated with acetazolamide. E. LABARCA, H. CROXATTO. *(Laboratorio de Fisiología, Instituto Pedagógico. Universidad de Chile).*

Modulation of spindle discharges during motor stimulation. CARLOS EYZAGUIRRE. *(Laboratory of Neurophysiology, Catholic University of Chile). (Aided by grants from the Rockefeller Foundation and the Fundación Gildemeister).*

Modulation of sensory discharges during centrifugal spindle activation was studied in toads. Single nerve fibres were stimulated in the ventral roots and sensory discharges recorded from single dorsal root fibres. Muscles were critically curarized in order to avoid extrafusal mechanical effects.

During the rising phase of an intrafusal twitch and at the beginning of intrafusal tetani a dynamic effect is observed. Static effects are observed a few seconds after the onset of tetanic contractions. Stimulation of the slow intrafusals produce static effects exclusively. Dynamic and static effects refer to the sensory acceleration patterns. Also, sensory discharges can be facilitated by adjusting the basal resting tension and the number of efferent motor stimuli. After prolonged stimulation the frequency of the sensory discharges declines presumably due to infrafusal fatigue.

The sensory discharges can be "driven" at frequencies which are identical to that of the delivered efferent impulses. This phenomenon was obtained only during stimulation of the fast intrafusals and it is probably produced by discrete oscillations of the intrafusal fibres even at high stimulating frequencies.

Simultaneous activation of fast and slow intrafusal systems produces sensory frequency changes which are not significantly different from changes obtained after independent stimulation of the systems. This occlusion is also produced by combined stimulation of several large or small axons. This phenomenon is interpreted as being produced by multiple polyneural innervation of single intrafusal fibre and by branching of the sensory libres which may supply more than one intrafusal bundle.

Efferent pulses to the fast intrafusal fibres determine a short sensory burst followed by a pause of the sensory discharger. This effect is blocked by increasing the resting tension or by simultaneous activation of the slow intrafusals.

The fast and slow intrafusal junctions are more resistant to the action of curare than their extrafusal counterpart.

It is concluded that many features of central reflexes are also present at the peripheral level. This fact may be of interest in the understanding of the servomechanisms responsible for posture regulation.

August 13, 1957

Evolution in the mesophragmatic group of the *drosophilus genus.* D. BRNCIC AND S. KOREFF. *(Instituto de Biologia "Juan Noé", Universidad de Chile).*

September 10, 1957

Physiologic adaptation to amphybiosis in *syciases sanguineus.* F. VARGAS AND I. CONCHA. *(Centro de Investigaciones Zoológicas, Universidad de Chile).*

Action of synthetic oxcytocin on urinary sodium and water excretion. F. ROZAS, H. CROXATTO AND NAVARRETE. *(Laboratorio de Fisiologia, Universidad Católica).*

October 8, 1957

Action of potassium on electroplates of *"Electrophorus electricus".* M. ALTAMIRANO. *(Physiology Department Columbia University).*

Variations in nucleic acid content of liver spleen and kidney of sarcoma bearing rats. M. PLAZA DE LOS REYES, P. MATUS, N. PÉREZ AND P. RUIZ. *(Instituto de Química Fisiológica y Patológica. Universidad de Chile).*

October 22, 1957

Effects of "Hemopoietine" on iron turnover in fasted animals. G. HODGSON, D. YUDILEVICH, M. PERRETA AND I. ESKUCHE. *(Sección Biológica, Laboratorio de Física Nuclear Pura y Aplicada, Universidad de Chile).*

The plasma iron turnover in rabbits fasted for four days, drops to 511 ug/1r (Normal 106 ug/h) and the % of Fe^{59} in erythrocytes at 24 hrs. in these animals diminishes from 43 % to 12,5 %. Injection of extracts of boiled plasma from phenylhydrazine treated rabbits ("Hemopoietine") increases plasma iron turnover and Fe^{59} uptake in erythrocytes to normal values. In rats fasted for 4 days injections of similar extracts increases Fe^{59} in erythrocytes (at 20 hrs.) from 5 % to 38 %.

The use of animals as a bio-assays for hemopoietine is discussed.

Renin potency of rats with high blood pressure due to D.O.C.A. adminis-

tration. H. CROXATTO AND T. BLAMEY. *(Laboratorio de Fisiología, Universidad de Chile).*

November 12, 1957

The effect of procaine and decamethonium on the contractile response to adrenaline and nor-adrenaline of denervated skeletal muscles. J. ZIELENIEWICZ, P. VENTURA-JUNCÁ, N. MIMICA AND E. RÍOS. *(Laboratorio de Neurofisiología "Gabriela G. Gildmeister", Universidad Católica)*

The blocking effect of d-Tubocurarine on the adrenaline and nor-adrenaline contractile response of denervated skeletal muscles (Luco and Sánchez, International Symposium of Curare, in press), suggested the possibility that other cholinergic blocking agents might have the same effect.

Unselected adult cats were used. The facial nerve of one side was aseptically severed from 8-10 days before the experiment. The superior cervical ganglion and the adrenal glands were acutely removed. The movement of denervated ear were recorded on a kymograph by isotonic heart level. The drugs: adrenaline, nor-adrenaline (Winthrop), decamethonium (Syncurine, Burroughs Wellcome & Co.) and procaine (Winthrop), were administrated into the carotid artery of the same side.

The doses of adrenaline and nor-adrenaline that elicited 40 to 50 % of the maximal response were used (from 0,25 to 2,5 micrograms). Once the response was regular, the blocking agents were injected intra-arterially. The contractile responses due to adrenaline and nor-adrenaline were blocked for some minutes by a previous administration of procaine (from 2 to 10 mg) and decamethonium (from 5 to 10 micrograms). Procaine also diminished the spontaneous activity of denervated muscles.

November 26, 1957

Effects of oxytocin on high blood pressure produced by D.O.C.A. in rats. H. CROXATTO AND G. BUSTAMANTE. *(Laboratorio de Fisiología, Universidad Católica).*

Acute intoxication with polycyclic hydrocarbon carcurogens. P. SHUBIK AND G. DELLA PORTA. *(Department of Oncology, Chicago Medical School).*

PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

June 12, 1958

Tumors of the ovary grafted in the spleen and progesterone. E. FELS. *(Instituto de Maternidad "Prof. Dr. A. Peralta Ramos", Buenos Aires.*

The effect of progesterone on the tumors of the ovary grafted in the spleen has been studied, proving that this hormone has no action on the tumor. This negative effect of the progesterone in open contrast with the results obtained when employing estrogens and androgens, can be explained because this hormone cannot suppress or restrain the gonadotrophin function of the hypophysis, which is responsible for the formation and growth of the tumors.

Isolation and purification of yeast fumarase. G. FAVELUKES Y A. O. M. STOPPANI. *(Instituto de Química Biológica, Facultad de Ciencias Médicas de Buenos Aires).*

By autolysis of baker's yeast acetone powder in neutral phosphate buffer, cell free, non-particulate preparations of fumarase can be obtained. The crude extracts can be purified about 12 times by treatment with calcium phosphate gel and succesive precipitations with ammonium sulphate, acetone and ammonium sulphate. Baker's yeast fumarase has the following properties: *a*) is stable at pH 7, *b*) inactivates irreversibly at pH below 6 or above 9; *c*) precipitates between 40 and 60 % saturation with respect to ammonium sulphate and with acetone between 38 and 50 % (v/v) at pH 7.0, and *d*) at low ionic strenght is selectively absorbed by calcium phosphate and alumina gels.

Diego blood factor investigated in indians of the "Quebrada de Humahuaca". J. L. SCARO. *(Instituto de Biología de la Altura. Universidad Nacional de Tucumán. S .S. de Jujuy).*

Diego blood factor was investigated in a group of 74 scantily mixed quechua indians living in the Quebrada de Humahuaca (Northeast-Argentina). 2.7 % cent of positive cases was found. Simultaneous determinations of 0 and Rh groups showed 98.7 and 100 per cent, respectively, of positive cases.

Water absorption in the toad's bladder. JULIA URANGA Y G. QUINTANA. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires).*

Water volume reabsorbed in two hours in the toad's bladder was measured.
I) Well hydrated toads reabsorbed 2.8 $\pm$ 0.3 ml of the water stored in their bladder.

2) Dehydrated toads, who had lost 11 ± 0.9 % of their weight reabsorbed 5.5 ± 0.4 ml of water.

3) Vasopressin and toad's neurohypophysis increase the absorption of the water contained in the bladder, but oxytocin has no effect.

4) No reabsorption of water was observed in the isolated bladder.

5) Toads with water in their bladder lived longer in a hypertonic solution than toads with an empty bladder.

July 3, 1958

Action of piribenzamine on the circulating eosinophils of the guinea pig. J. L. SCARO, E. SLEIBE Y H. CHIODI. *(Instituto de Biología de la Altura, Universidad Nacional de Tucumán, S. S. de Jujuy, Argentina).*

Piribenzamine injected intraperitoneally to guinea pigs, produced in a four hour period, a significant fall of the circulating easinophils, but without any change in the ascorbic acid content of the adrenals. Thyroidectomy prevented the eosinophilopenic reaction of the piribenzamine but not that of the ACTH. The injection of 3-5-3' triiodothyronine restored eosinophilopenic reaction abolished by thyroidectomy.

Toxicity of dichlorodiphenyldichloroethane (D.D.D.) in the rat. N. J. CARLISKY. *(Instituto de Biología y Medicina Experimental). (Instituto de Fisiología de la Facultad de Ciencias Médicas).*

The toxicity of D.D.D. has been studied with white female adult rats, previously kept fasting during 24 hours. Then one group was kept fasting 84 hours more, while another group was fed "ad libitum". The values of D.L. 50, established directly through experimentation, were of 500 mg per 100 g weight in the first and the second group. The values of D.L. 100 were 1 000 mg per 100 g weight for the group that had been kept fasting and 1 250 mg per 100 g weight for the rats fed "ad libitum". After 84 hours, all the rats that had been kept fasting, to whom 500 mg (or more) per 100 g weight were administered, died, while in the other group of rats (that had been fed "ad libitum") no death was recorded subsequently among those animals that had survived after the said period.

Reabsorption of water in the toad's kidney. JULIA URANGA. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires).*

The inulin clearance in the toad was measured, studying the possible modifications produced by the activity of vasopressin and neurointermediate lobe.

1) No alteration in the glomerular filtration rate was observed by the action of these hormones.

2) The glomerular filtrate was higher in Summer than in Winter..

3) No sexual difference was observed.

Electron microscopy of the pancreas in the alloxan diabetes of the rat.
P. E. LACY Y A. F. CARDEZA. *(Departamento de Patología, Universidad de Washington, St. Louis, Missouri, EE. UU.)*

The electron microscopic study of the islets of Langerhans of Wistar male rats injected intravenously with 50 mg of alloxan per kg of body weight, was carried out.

The animals were fasted during 65 hours and were killed 5, 15, 30 minutes, 1, 2, 4, 8, 24 hours, 4, 7 and 9 days after the injection.

Numerous agranular cells were observed at the periphery of the islets in the control rats. These cells were not found in normal fed rats.

Five minutes after alloxan administration, numerous vacuoles were observed in the cytoplasm of some beta cells. They originated from the ergastoplasmic membranous sacs. Vacuolization of beta cells are progressive and at 24 hours almost all the beta cells become necrotic. Numerous macrophages appear into the islets phagocytizing debris of necrotic cells. Normal secretory beta granules are still present in the necrotic cells and also in the phagocytized debris into the macrophages.

The cytoplasm of the capillary endothelium is thicker than normal and show finger-like projections extending through the capillary lumen. The islets of diabetic rats between 4 and 9 days are composed by alpha cells in their majority and small number of degranullated beta cells. The acinar cells at this time contain numerous lipid droplets in their cytoplasm.

Study of sexual chromatine in malignant neoplasms of the male breast.
EUGENIA SACERDOTE DE LUSTIG *(Instituto de Oncología "Angel H. Roffo").* BEATRIZ GARCÍA TUÑÓN DE POGO *(Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Buenos Aires).*

The sexual characteristics of the nuclei of 6 malignant neoplasms of the male breast have been studied. All the tumours show sexual chromatin within the limits which belong to the sex of the patients $x = 3{,}45\ \% \pm 0.22$, there existing no relation between the clinical malignacy and the appearance of its anomalies.

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	Castellano	*Inglés*
metro	m	m
centímetro	cm	cm
milímetro	mm	mm
micrón	μ	μ
milimicrón	$m\mu$	$m\mu$
Angström	Å	Å
microgramo	μg	μg
gama	γ	γ
hora	h	hr
minuto	m	Min
	s	sec

	Castellano	*Inglés*
milisegundo	ms	msec
litro	l	l
centímetro cúbico	cm^3	cc
mililitro	ml	ml
kilogramo	kg	kg
gramo	g	gm
miligramo	mg	mg
miliequivalente	mEq	mEq
Curie	c	c
Milicurie	mc	mc
Microcurie	μC	μC
por ciento	%	%

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